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THE EFFECT OF THE SUGAR CONCENTRATION ON CONIDIAL SIZE OF SOME SPECIES OF HELMINTHOSPORIUM[1, 2]

EDWARD S. ELLIOTT

(Accepted for publication June 24, 1949)

As part of a more extensive study of factors influencing the sporulation of fungi, the effect of the sugar concentration in the medium on the size of conidia of some species of *Helminthosporium* has been investigated.

Taxonomic descriptions of spore size as given by different workers are often found to vary markedly. Pammel, King, and Bakke (5) have described *Helminthosporium sativum* as having conidia, in nature, which measure 105 to 130 μ in length and contain 7 to 14 cells. Dosdall (2) described the conidia of this species as being 56 to 83 μ long, while Stakman (7) found the conidia of three isolates to vary in average length from 35 to 60 μ.

Undoubtedly, some of the reported variation is due to biologic forms of this species, as has been shown by Christensen (1). He found at least four biologic forms of *Helminthosporium sativum* which differed in their degree of pathogenicity, in physiological characteristics in culture, and in the size and number of septations of conidia.

Lack of uniform methods of culturing has probably contributed more to these differences than any other factor. Sherbakoff (6) found the conidial size of several species of *Fusarium* to vary widely when cultured on different media.

This paper reports the effect of the quantity of sugar in the medium on the size of conidia produced by some species of *Helminthosporium*. It is believed that these investigations will explain, in part, why such variation in spore size has been reported by different investigators. The need for more standardized culture methods is indicated.

MATERIALS AND METHODS

The cultures of *Helminthosporium* used in this work included *H. victoriae* M. and M., isolated from Victland oats grown in northern West Virginia, *H. sativum* Pam., King, and Bakke, *H. turcicum* Pass., *H. oryzae* Breda de Haan, and *H. sigmoideum* var. *irregulare* Cralley and Tullis. All produced conidia readily on several media.

Three kinds of media were used: malt extract, a glucose-casein hydrolysate medium as described by Leonian and Lilly (4), and a glucose-nitrate

[1] This paper is based on work done for Camp Detrick, Maryland, under contract No. W-15-035-CE-167 with West Virginia University.

[2] Published with the approval of the Director of the West Virginia Experiment Station as Scientific Paper No. 404.

medium consisting of sodium nitrate ($NaNO_3$), 2.0 gm.; potassium phosphate (K_2HPO_4), 1.0 gm.; potassium chloride (KCl), 0.5 gm.; magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.5 gm.; ferrous sulfate ($FeSO_4 \cdot 7H_2O$), 0.1 gm.; double distilled water to make 1000 ml.; glucose, variable. All media contained 20 gm. agar per liter.

The sugar content of the media was varied but the nitrogen source and other components remained constant, except for the malt extract medium. The necessary amount of malt extract was added to double distilled water and the entire medium was diluted to secure the various sugar concentrations. The desired glucose content of the other media was secured by preparing the sugar solution separately and adding the proper amount to each Petri dish. Water was then added to make the total volume 24 ml. The media were sterilized for 15 min. at 15 lb. pressure. Small pieces of mycelium and spores used for inoculum were taken from cultures growing on agar slants in test tubes.

The cultures were incubated at 25° ± 1° C. in diffuse light. They were allowed to reach maturity before the conidia were measured; this generally required 20 to 30 days. Conidia were produced sooner on media containing low concentrations of sugar. A camera lucida and a calibrated scale were used to measure the length of conidia.

The length of conidia was correlated with the number of cells in the conidia. Both measurements (length and number of cells) were used in most cases to determine the average size of conidia, one being used as a check on the other. Width of conidia also varied, but no attempt was made to correlate this with the length and number of cells.

Since the object of this work was to determine the variation in the size of conidia produced on media containing different quantities of sugar, only 25 conidia were measured. The average length of numerous groups of 25 conidia taken at random from the same culture varied but little. Where there was doubt that the average length of 25 was not in close agreement, 50, 75, or 100 conidia were measured. All the conidia in each microscopic field under the high power objective were measured unless the number exceeded the desired total of 25. If less than the desired number were present in the field, other fields selected at random were used.

Since the differences obtained in spore size were so great, it was not considered necessary to treat the data statistically.

RESULTS

Helminthosporium victoriae. Preliminary experiments with malt extract medium showed that the length and number of septations of the conidia were inversely proportional to the concentration of malt extract. Measurement of 100 conidia from each medium substantiated these preliminary observations. The 2 per cent malt extract agar yielded conidia which meas-

ured 22.2 (10–60) μ long,[3] with 2.8 (1–7) cells[3]. Conidia from 0.5 per cent malt extract agar medium measured 35.7 (15–50) μ and contained 5.1 (2–8) cells. Those from 0.1 per cent malt extract agar medium measured 59.1 (43–78) μ and contained 7.4 (5–9) cells.

The results of the preliminary studies were confirmed by measuring conidia of *Helminthosporium victoriae* produced on three different solid agar media—malt extract, casein hydrolysate, and glucose nitrate—each

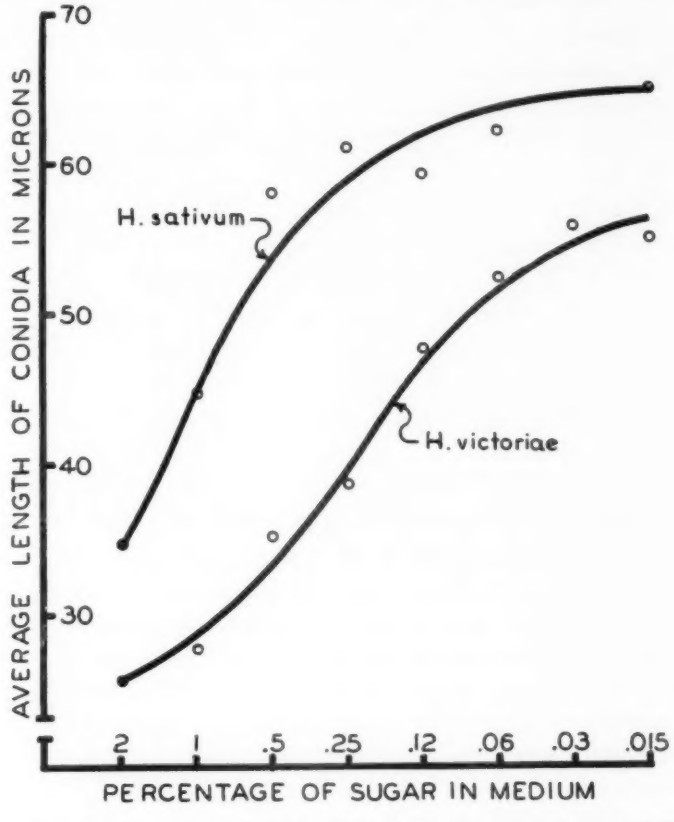

FIG. 1. The trend in average length of *Helminthosporium sativum* and *H. victoriae* conidia from cultures on glucose-nitrate medium containing different sugar concentrations.

having 8 different sugar concentrations ranging from 2 per cent to 0.015 per cent. The average length of conidia varied from a minimum of 25.8 μ on 2 per cent sugar to a maximum of 56.0 μ on 0.03 per cent sugar.

Each of the three media yielded conidia which averaged about the same length providing there was an equal amount of sugar in each. The close correlation between the average length of conidia and the sugar concentration of the glucose-nitrate medium is illustrated in figure 1. A similar correlation between the average number of cells and the sugar concentration for each medium is shown in table 1.

[3] Average, minimum, and maximum measurements and cell count indicated.

Helminthosporium sativum. Malt extract and glucose-nitrate agar media, having 8 sugar concentrations ranging from 2 per cent to 0.015 per cent, were used for cultures of *H. sativum.* When the average length of conidia was determined for each concentration it was found that the trend in average length was similar to that described for conidia of *H. victoriae.* As in *H. victoriae* there was a correlation between the length of conidia and the number of cells that they contained. The average length of conidia produced on different amounts of sugar in the basal glucose-nitrate medium is shown in figure 1. The average number of cells in the conidia were similar (Table 1) for the same dilution of each medium.

TABLE 1.—*The effect of various amounts of sugar in the media on average number of cells contained in conidia of Helminthosporium victoriae and H. sativum*

Percentage of sugar in medium	Average number of cells[a] in conidia			
	H. victoriae		*H. sativum*	
	Glucose nitrate	Malt extract	Glucose nitrate	Malt extract
2.0	2.8 (1– 4)	3.4 (1– 6)	4.1 (1– 9)	4.2 (2– 7)
1.0	3.8 (2– 8)	3.8 (2– 6)	5.6 (4– 9)	5.0 (2– 9)
0.5	4.9 (4– 9)	4.5 (2– 7)	6.7 (5– 9)	6.2 (2– 9)
0.25	5.2 (3– 7)	5.6 (3– 8)	7.1 (5– 9)	6.8 (3–10)
0.12	6.3 (4– 8)	7.1 (5–10)	7.0 (4– 9)	7.6 (4–11)
0.06	7.1 (5– 9)	7.2 (5– 9)	7.4 (6–10)	7.7 (6– 9)
0.03	7.3 (6– 9)	7.8 (7–10)	.....	7.7 (6– 9)
0.015	7.3 (4–10)	7.7 (5–10)	7.6 (6–11)	7.3 (5–10)

[a] Figures in parentheses give minimum and maximum number of cells in conidia.

Since the media containing 2 per cent sugar produced conidia of the minimum average size, another series of glucose-nitrate media containing larger amounts of sugar was used to find if more sugar would further reduce the average size. Media containing 2 per cent, 8 per cent, and 16 per cent sugar produced conidia which averaged 35.0 μ, 32.8 μ, and 33.0 μ, respectively. A further increase in sugar above 2 per cent did not change the minimum average length to any extent but tended to reduce the total number of conidia.

Other species. Cultures of *Helminthosporium sigmoideum* var. *irregulare* grown on glucose-nitrate medium of 8 different sugar concentrations varying from 2 per cent to 0.015 per cent produced conidia which followed the trend described for *H. victoriae* and *H. sativum.* Since conidia of this species are small and relatively uniform in length, the variation in average size from high to low sugar concentrations was not so great as in the previously studied species. Although there was some indication that conidia of *H. turcicum* varied in average length depending upon the amount of sugar in the medium, the results were not conclusive. A marked decrease in average size of conidia of *H. oryzae* was found only on media containing more than 4 per cent sugar. The average size was reduced from 93 μ on 4 per cent sugar to 59 μ on 16 per cent sugar.

DISCUSSION

In these experiments, conidial length of four species of *Helminthosporium* was found to be regulated by the quantity of sugar in the medium. The average length of conidia was inversely proportional to the amount of sugar available. The range in sugar concentration over which the variation in average length occurs is not the same for each species. An increase in the amount of sugar beyond that required to produce conidia of the minimum average length, or a decrease beyond that required to produce the maximum average length, serves only to reduce the total number of conidia. Under the cultural conditions provided, time of incubation had no apparent effect on the size of conidia produced. Mycelial growth increased with increasing quantities of sugar, which may partially explain the reduction in size of conidia if we assume that food materials are utilized for vegetative growth rather than sporulation when an abundant supply is available. Whether the size and number of conidia produced in nature on the host plant is related to the sugar content at various stages of growth of the host is a problem which would require further study.

The number of cells contained in the conidia was directly proportional to the length of conidia. A study of the number of cells alone would give results which would closely approximate those obtained by measuring spore length.

Horne and Mitter (3) in studying some species of *Fusarium* arrived at similar conclusions regarding the effect of glucose on the sporulating capacity and the frequency of septation. They have reported that by increasing the glucose concentration either the sporulating capacity was reduced or the average septation number was lowered, or both. With one strain of *F. culmorum*, spore production gradually fell off as the glucose increased from an initial concentration of 2 per cent to a concentration of 20 per cent. The average septations in this case remained unaltered. With another strain of *F. culmorum* both spore production and the average septation declined within this range. The number of septations in spores of *F. polymorphum* declined sharply as glucose concentration increased from 2 per cent to 8 per cent.

The obvious differences in the size of conidia formed on media having different sugar contents indicates one reason, at least, why taxonomic descriptions of spore size are often not in agreement. This variation probably is not peculiar to the genus *Helminthosporium*, but may also occur in other fungi.

These data indicate that whenever size of conidia formed in culture is reported, the exact composition of the medium and other cultural conditions should be given. This seems particularly true for groups in which the species or varieties may be separated on the basis of conidial measurements.

SUMMARY

The conidial size of four species of *Helminthosporium* in culture was influenced by the quantity of sugar in the medium. Within limits, the length of conidia was inversely proportional to the amount of sugar available to the fungus. The numbers of cells in the conidia were generally found to be directly proportional to the length of conidia.

H. victoriae produced conidia of maximum average length on media containing 0.03 per cent sugar, and conidia of minimum average length on media containing 2 per cent sugar. The average length on 0.03 per cent sugar was more than double the average on 2 per cent sugar.

H. sativum produced conidia of maximum average length on media containing 0.12 per cent sugar. Conidia that were approximately half that size, the minimum average length, were produced on media containing 2 per cent sugar.

The average size of conidia of *H. oryzae* was markedly decreased only on media containing more than 4 per cent sugar.

Since conidia of *H. sigmoideum* var. *irregulare* are small and relatively uniform in length, the variation in average length from high to low sugar concentrations was not great, but it followed the trend already described.

No conclusive results were obtained to show that conidia of *H. turcicum* vary in average size on media of different sugar concentrations.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT, USTILAGO NUDA, IN BARLEY[1]

C. W. SCHALLER[2]

(Accepted for publication June 30, 1949)

INTRODUCTION

Loose smut of barley, caused by *Ustilago nuda* (Jens.) Rostr., is an important disease in many barley-producing areas, particularly in the humid and subhumid areas of production. While not so prevalent as some diseases, the lack of easily applied control measures increases its importance in areas where it does occur. The reduction in yield is approximately in direct proportion to the percentage of smutted plants (13). Although loose smut can be controlled by treating the seed with hot water, this method is difficult and hazardous to use and often reduces germination of the seed. Resistant varieties offer the best practical means of control.

Shands and Schaller (15) reported the reaction of a number of spring barley varieties and listed several with a high type of resistance. Poehlman (7) found good resistance in several winter barleys. Physiological specialization has been shown to occur within the loose smut organism (6, 11, 12), making it desirable to have more than one available source of resistance.

Equally important in a breeding program is an understanding of how resistance is inherited. Resistance controlled by a single gene is more readily utilized than that of a complex nature. A favorable linkage of resistance with some agronomically desirable character would greatly facilitate the breeding of resistant varieties. Shands (14) reported a linkage between the genes conditioning the loose smut resistance of Trebi and the stem rust resistance of Chevron. Selections are now available which carry these genes in the coupling phase. Early generation testing for loose smut resistance can be reduced by selecting stem rust resistant segregates.

Shortly after the present investigation was started, Livingston (5) reported that the resistance of Trebi was conditioned by a single gene. He found a similar gene in a selection of *Hordeum deficiens* (*Hordeum distichum* var. *deficiens* Steud.), and a weak gene in Missouri Early Beardless. There was no evidence of linkage between the genes for resistance and those for hoods or kernel row number.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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The writer wishes to express his appreciation to Drs. H. L. Shands and J. G. Dickson for their generous aid and guidance throughout the course of this investigation.

In a series of crosses, Zeiner (16) found indications that single genes conditioned immunity, resistance, and moderate susceptibility. Resistance appeared to be dominant over susceptibility. The genes for resistance and moderate susceptibility were shown, in one cross, to be inherited independently. While not conclusive, Nahmmacher's work (6) suggested single gene differences between resistant and highly susceptible and between resistant and moderately susceptible varieties.

This paper deals with the genetics of resistance to loose smut in four varieties of barley. Varieties were selected which differed in their place of origin and in their level of resistance. These were crossed with susceptible varieties and, with one exception, in all possible combinations. Whenever the parents differed in morphological characters, the progenies were classified and tests of associations with resistance made.

MATERIALS AND METHODS

Hybrid populations obtained from crosses with six barley varieties were used in this study. The loose smut reactions of these varieties, as shown in table 1, varied over a wide range. Newal and Colsess IV were highly susceptible, and selection X173-10-5-6-1 was moderately susceptible under the conditions of these tests. Jet, in which no infected plants have been found, and Trebi were highly resistant. Dorsett, with an average infection of 12.9 per cent, was considered resistant, but lacked the high type of resistance shown by Jet and Trebi.

TABLE 1.—*Reactions of parental varieties when inoculated artificially with Ustilago nuda*

Variety	C.I. No.[a]	Years tested	Tests[b]	Plants	Average infection
		No.	*No.*	*No.*	*Pct.*
Newal	6088	5	30	1584	70.5
Trebi	936	5	31	1323	1.3
Colsess IV	5979	2	21	1092	84.9
Jet	967	2	16	299	0.0
X173-10-5-6-1		2	17	1196	43.6
Dorsett	4821	3	23	1275	12.9

[a] C.I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.
[b] A test represents the progenies from 2 inoculated heads.

Newal and selection X173-10-5-6-1 are white, 6-rowed, smooth-awned varieties, the former of commercial importance in Canada, the latter a Wisconsin selection from the cross [Oderbrucker (C.I. 4666) × Lion (Wis. 117)] × Oderbrucker (C.I. 4666). Trebi, a variety of commercial importance, has been used as a source of resistance to loose smut in many breeding programs. Colsess IV, developed by Robertson (8), carries the lethal seedling character xantha, a marker for linkage group VI, in a heterozygous condition. Both the heterozygous and homozygous green lines of this

stock were used. Jet is a black, naked-kerneled, 2-rowed variety of Abyssinian origin, and Dorsett is a black, 6-rowed variety from Manchuria.

The "needle" method of inoculation, as described by Shands and Schaller (15), was used throughout this investigation. A small rubber bulb containing dry chlamydospores was attached to a hypodermic needle (20-gage). Using the tip of the needle to pierce the lemma of each flower on the spike, the dry spores were introduced directly into the flower by a gentle pressure on the bulb. The small flowers at the terminal and basal joints of the rachis were removed before inoculation. The inoculations were made in the field, except in a few cases where the F_1 generation was inoculated in the greenhouse. Inoculations 1 day after anthesis of the median florets gave the most satisfactory results. The plants from the inoculated seed were grown either in the field or in the greenhouse. The progeny of each inoculated head was handled as a separate unit.

The inoculum was collected originally from a single smutted head occurring naturally in the susceptible variety Newal, on which it was increased and maintained. The spores were freshly gathered, except in a few cases where they had been stored 2 or 3 months at 4° C. In all cases a representative sample of the spores was germinated to determine their vigor and to ascertain the purity of the species.

Hybrid material was tested in the F_1, F_2, F_3, F_4, and backcross generations. Since some genetically susceptible plants may escape infection, the F_2 does not permit a satisfactory genetical analysis. Classification of F_2 plants, based on the behavior of their progenies in F_3 rows, and the F_2 progeny tests of backcrossed plants formed the basis for the genetical interpretation of the genes involved. Limited tests of F_4 lines were made to check on the F_3 classification.

Because loose smut is floral-infecting, it is necessary to introduce the spores into the flowers of the plants producing the embryos of the generation to be tested. The reaction of the F_1 generation was obtained by inoculating the flowers of the female parent 24 hr. after the introduction of the pollen from the male parent and then growing the plants from the inoculated seed. Tests on the F_3 lines were made by inoculating the flowers of three heads of each F_2 plant taken at random from disease-free F_2 populations. The three heads from each plant were kept separate and grown in individual rows representing three replications. The average infection of the three rows was used in determining the reaction of the F_2 plant. Since the plants varied in time of flowering, all inoculations could not be made on the same day. Flowering time extended over a period of 10 to 14 days. An analysis of variance on the percentage of infection in the parental varieties inoculated over the 10- to 14-day period showed that the variation in infection was not significant. All F_3 lines of one replication were grown at the same time, usually one replication in the greenhouse and two in the field, except in a few cases where all three replica-

tions were grown in the greenhouse. Parental checks were included at regular intervals.

The material from the backcross was handled in a similar manner; *i.e.*, flowers on three heads of each backcross F_1 plant were inoculated. The F_4 tests were made by inoculating the flowers of a single head from a few plants in the noninoculated F_3 lines.

At harvest time, the plants were classified as either healthy or smutted. Plants with only one smutted tiller were classified as susceptible. The total number of plants and the number of smutted plants were recorded and the percentage of smut calculated.

Whenever parents of a cross differed in one or more morphological characters, segregations of these characters were observed. Classification was made on F_2 plants and, in general, verified in the F_3. Backcross plants and their progeny were classified also. Of particular interest was the association or lack of association of the morphological characters with smut reaction.

EXPERIMENTAL RESULTS

Smut Infection in the F_1 and F_2 Generations

Since infection occurs at flowering time, the infecting hyphae in order to reach the embryo must penetrate through floral tissues which may differ genetically from that of the embryo tested. It was necessary, therefore, to ascertain the effect of this maternal tissue on infection, and also whether resistance is determined by the genetic constitution of the floral tissue or that of the developing embryo.

TABLE 2.—*Reactions of the F_1 and F_2 generations of various barley crosses and their reciprocals when inoculated with Ustilago nuda*

Cross	Generation	Total plants	Infected plants	
		No.	*No.*	*Pct.*
Newal × Trebi	F_1	53	1	1.9
Trebi × Newal	F_1	61	0	0.0
Newal × Trebi	F_2	404	34	8.4
Trebi × Newal	F_2	121	8	6.6
Colsess IV × Jet	F_1	56	0	0.0
Jet × Colsess IV	F_1	28	0	0.0
Colsess IV × Jet	F_2	281	35	12.4
X173-10-5-6-1 × Dorsett	F_1	25	9	36.0
X173-10-5-6-1 × Dorsett	F_2	266	132	49.6
Dorsett × X173-10-5-6-1	F_2	138	64	46.4
X173-10-5-6-1 × Newal	F_2	195	125	64.1

The smut reactions of the F_1 and F_2 generations of the various crosses and their reciprocals are given in table 2. The amount of infection for the reciprocal crosses was the same. The 1.9 per cent infection in the F_1 of Newal × Trebi was no greater than would be expected for the resistant parent Trebi (Table 1). The lack of infection in the F_1 generation of

the crosses Newal × Trebi and Colsess IV × Jet must be attributed to the resistance of the heterozygous embryos, as the floral tissue of the female parents, Newal and Colsess IV, was susceptible.

The infection in the F_2 generations of the same crosses and their reciprocals (Table 2) showed that the fungus was able to become established in F_2 embryos which were surrounded by maternal tissue having the F_1 complement of genes for resistance and susceptibility. In this case, the embryos were surrounded by maternal tissue which was heterozygous for resistance. As was shown by the lack of infection in the F_1 generation, the heterozygous condition of the embryo gave the developing plant complete protection.

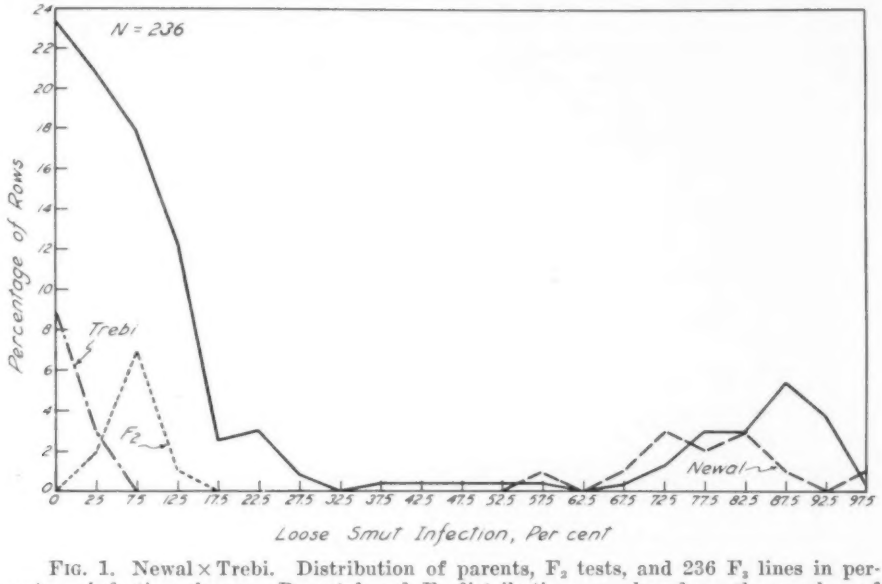

FIG. 1. Newal × Trebi. Distribution of parents, F_2 tests, and 236 F_3 lines in percentage infection classes. Parental and F_2 distributions are based on the number of tests rather than the percentage of rows in each infection class.

Lang (4) and Ruttle (12) have demonstrated that the barley loose smut fungus is able to reach the embryo by two different routes. The infecting hyphae can either follow the same route as the pollen tube or penetrate directly through the ovary wall. It is conceivable that resistant floral tissue might block direct penetration through the ovary wall, which would reduce the amount of infection in the F_2 generation. As will be pointed out later, the infection in the F_2 generation was slightly lower than that expected. There is a need for histological investigations on the mode of penetration when the floral tissue is resistant.

Inheritance of Resistance to Loose Smut in Hybrids with Trebi

Trebi was highly resistant and Newal highly susceptible when inoculated artificially. The smut reactions of the F_1 and F_2 generations (Table

TABLE 3.—*Distribution of parent rows, F_1 and backcross progenies for loose smut infection by percentage classes for crosses indicated*

Parent or cross	Percentage classes																				Total number of rows
	0	0.1–5	5–10[a]	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
1943																					
Newal													1	0	1	3	2	3	1	0	1	12
Trebi	9	3																				12
Newal × Trebi	55	49	42	29	6	7	2	0	1	1	1	1	1	0	1	3	7	7	13	9	1	236
1945 and 1946																						
Newal									1	0	1	2	0	1	2	0	0	1	0	0	2	10
Trebi	4	3	1																			8
F_1 (Newal × Trebi) × Newal	3	7	10	4	2	2	0	0	0	2	2	1	3	1	3	3	1	4	3	4	2	57
1946																						
Colsess IV																	2	3	1	3	2	11
Jet	7																					7
Colsess IV × Jet	27	6	13	7	8	11	2	1	0	0	0	1	2	1	1	2	3	3	1	0	2	91
Colsess IV × F_1 (Colsess IV × Jet)			6	6	13	13	7	4	2	1	0	3	3	4	7	11	6	13	7	5	5	116
Trebi × Jet	56	15	3	7	1	2	2	0	0	0	1	0	1	0	1							89
1943																						
X173-10-5-6-1							1	3	2	2	2	1	1									12
Dorsett		2	2	1	3	3	1															12
X173-10-5-6-1 × Dorsett	1	9	18	18	17	9	11	13	21	19	21	22	12	16	10	8	2	3	1			231
1946																						
Dorsett × Trebi	43	21	7	4	0	2	1	0	2	0	4	2	4	0	1	0	2	1	0	1	1	96
Dorsett × Jet	46	17	13	9	1	1	2	2	2	2	0	1	0	0	0	2	0	0	1	0	0	99

[a] 5.1 to 10.0 per cent inclusive.

2) indicated that the resistance of Trebi was dominant. No significant differences appeared between progenies of reciprocal crosses.

The smut infection of the parental varieties and 236 F_3 progenies by 5 per cent classes is given in table 3 and shown graphically in figure 1. The small number of lines between the 32 and 62 per cent infection classes divided the progenies into two groups, approximating a 3:1 ratio. Such a ratio would be expected if the resistance of Trebi was conditioned by a single gene.

In order to separate accurately the homozygous susceptible and the segregating lines, the F_3 lines with an average infection between 28 and 58 per cent were tested in the F_4 generation. Corresponding F_3 lines from noninoculated F_2 heads were used. The results, given in table 4, showed

TABLE 4.—*Comparison of percentage smut infection of F_2 lines intermediate in loose smut reaction with percentage infection in corresponding F_3 lines. Newal × Trebi*

F_2 plant number	Infection in F_3 generation	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Pct.	*No.*	*Pct.*	*Pct.*
12	28	4	4	0–8
24	29	4	6	0–11
126	30	3	6	0–11
40	39	4	49	30–58
123	40	4	82	62–95
155	48	5	79	68–93
96	51	3	76	67–90
125	58	4	66	42–88

that the F_3 lines with an average infection of 30 per cent or less were segregating for resistance, while those F_3 lines which had more than 30 per cent were homozygous for susceptibility. The average infection of all F_4 lines of the latter group was 70 per cent. Failure to obtain susceptible F_4 lines from the 3 segregating F_3 lines can be attributed to the small number of F_4 lines tested.

There were 190 F_3 lines with less than 30 per cent and 46 lines with more than 30 per cent infection. Although the infection range of the 46 susceptible lines was slightly greater than that for the susceptible parent (Fig. 1), the F_4 tests showed them to be homozygous for susceptibility. The average infection for the 46 lines was 80.7 per cent, compared with 77.5 per cent for Newal. The χ^2 test for goodness of fit to a 3 : 1 ratio gave a probability of 0.10 to 0.05.

No attempt was made to separate the homozygous resistant lines from those segregating for resistance, as considerable overlapping of the two groups would be expected on the basis of the distribution of the resistant parent and the F_2 tests (Fig. 1). Since resistance was conditioned by a single gene, the distribution of the segregating F_3 lines should approximate

that of the F_2. The distribution of the parental checks and the F_2 tests shown in figure 1 and subsequent figures are based on actual test numbers rather than on percentage of rows since their infection extended over only a small portion of the curve.

The plants from backcross of F_1 plants to the susceptible variety Newal were grown without inoculation, and the lines obtained tested for smut reaction. Results of the reciprocal crosses, the F_1 used either as the male or female parent, were combined because of their similarity. The distribution of the 57 backcross F_2 lines is given in table 3 and shown graphically in figure 2. A complete separation of the lines into two groups occurred at the 25 to 40 per cent infection level.

There were 28 lines in the low infection group and 29 lines in the high infection group which conformed with the 1:1 ratio expected when one gene conditions resistance. As expected, the distribution of the lines in

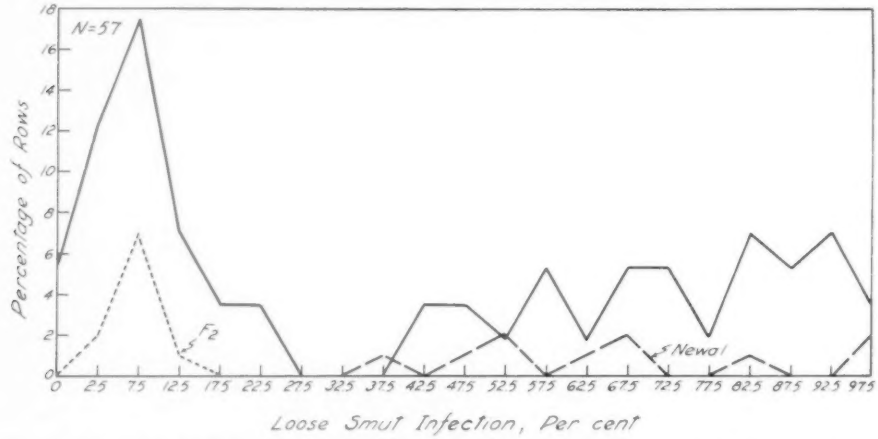

FIG. 2. F_1 (Newal × Trebi) × Newal. Distribution of the susceptible parent, F_2 tests, and 57 backcross lines in percentage infection classes. Parental and F_2 distributions are based on the number of tests rather than the percentage of rows in each infection class.

the low infection group conformed with the distribution of the F_2 tests (Fig. 2). The average infection of the F_2 tests was 7.5 per cent and for the 28 segregating lines 7.9 per cent. This was in close agreement, even though they were grown in different years. The spread of the lines in the high infection group was within the range of the susceptible variety (Fig. 2), and they can be considered homozygous for susceptibility. The average infection of this group was 73.8 per cent, for the Newal parent, 66.7 per cent. The wide range of infection in Newal and in the susceptible lines can be attributed to the fact that two years' results were combined. The level of infection for the two years differed considerably.

The results for the several generations of the cross Newal × Trebi and the backcross agreed very well with the ratios that would be expected if resistance of Trebi was conditioned by a single dominant gene. However,

one would normally expect a higher level of infection in the F_2 generation and in the F_3 rows from heterozygous F_2 plants. Part of this reduction can be accounted for by a slight selective mortality of infected plants caused either by low germination or weak germination followed by death of the seedlings. The F_3 families containing infected plants gave a lower percentage stand than those with no infection.

Infection groups	0	1.0 – 30	30.1 – 100
Average per cent stand	63	58	57

Selective mortality was also noted in the backcross lines.

Infection groups	1 – 30	30.1 – 100
Average per cent stand	58	52

Also, as was pointed out previously, the presence of resistant maternal tissue surrounding the developing embryos in heterozygous F_2 plants might possibly cause a slight reduction in infected plants.

Inheritance of Resistance to Loose Smut in Hybrids with Jet

Jet has maintained its high resistance throughout this investigation; no smutted plants appeared in any of the tests. Colsess IV was highly susceptible, averaging 84.9 per cent smutted plants. The F_1 plants were resistant when either variety was used as the female parent (Table 2). The absence of infection in the F_1 generation and the low amount in the F_2 generation suggested that the resistance of Jet was transmitted as a dominant character, and that the infection of the hybrids was determined by the nature of the embryo rather than by the floral tissue of the female parent.

The distributions of the parental varieties and 91 F_3 progenies for smut infection are given in table 3 and shown graphically in figure 3. The complete break in the curve at the 42.5 per cent infection class divided the progenies into two groups, 75 progenies with less than 37.5 per cent infection, and 16 with more than 47.5 per cent infected plants. This distribution suggested a 3:1 ratio, which would be expected if a single major gene for resistance was acting. The χ^2 test gave a probability of 0.20–0.10 for this ratio.

Since Jet was completely resistant, it can be assumed that the F_3 progeny rows from homozygous resistant F_2 plants would fall in the 0 per cent infection class. On the basis of the distribution of the F_2 tests (Fig. 3) and the distribution of the heterozygous backcross lines which will be discussed presently, very little overlapping of the homozygous and heterozygous F_3 rows would be expected. Considering the F_3 progenies with 0 per cent infection as homozygous resistant, those with infection up to 37.5 per cent as segregating, and those with more than 47.5 per cent as homozygous susceptible, the distribution fitted a 1:2:1 ratio with a probability of 0.50–0.20..

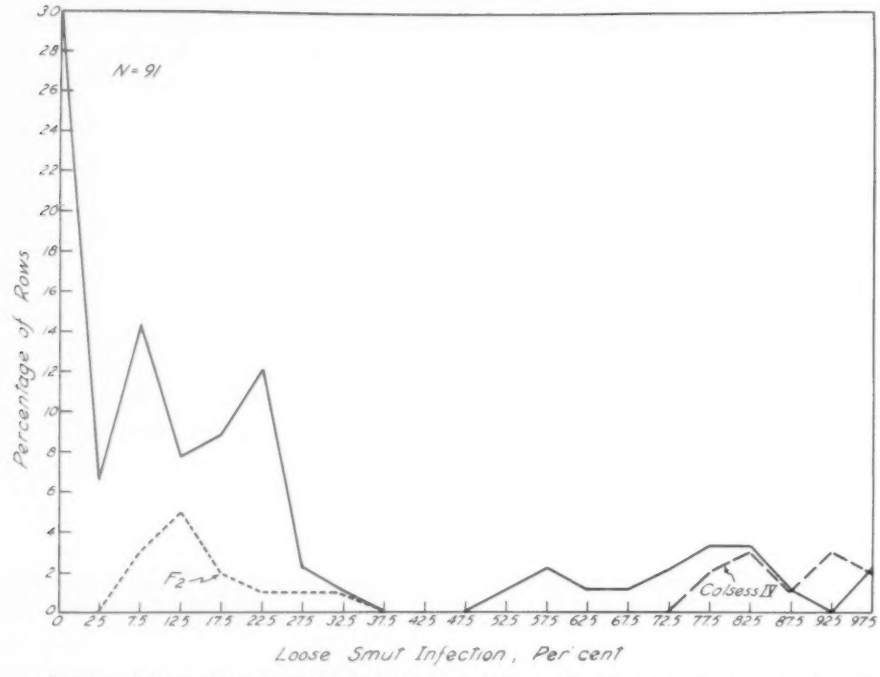

FIG. 3. Colsess IV × Jet. Distribution of Colsess IV, F_2 tests, and 91 F_3 lines in percentage infection classes. Parental and F_2 distribution are based on the number of tests rather than the percentage of rows in each infection class.

The distribution of 116 backcross F_2 progenies is given in table 3, and shown graphically in figure 4. The recurrent variety, Colsess IV, was used as the female parent. A complete break in the curve at the 47.5 per cent infection level separated the rows into two groups of approximately equal numbers. There were 52 lines with less than 47.5 per cent and 64 rows with more than 47.5 per cent infection. This suggested a 1:1 ratio

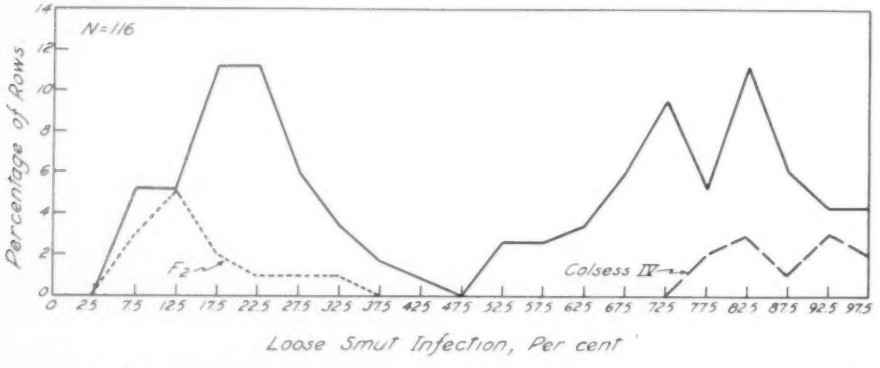

FIG. 4. Colsess IV × F_1 (Colsess IV × Jet). Distribution of Colsess IV, F_2 tests, and 116 backcross lines in percentage infection classes. Parental and F_2 distributions are based on the number of tests rather than the percentage of rows in each infection class.

of heterozygous and susceptible progenies, which would be obtained if a single dominant gene for resistance was present in Jet. The χ^2 test gave a probability of 0.50 to 0.30 for this ratio. The segregating rows averaged 20.8 per cent infection and the susceptible rows 80.0 per cent.

The various generations of the cross Colsess IV and Jet and the backcross gave distributions which agreed with the ratios expected if the resistance of Jet was conditioned by a single dominant gene.

The distribution of 89 F_3 progenies of the cross Trebi × Jet is given in table 3. Three rows had more than 45.0 per cent infection and were classed as susceptible. Since segregation occurred, the genes for resistance in Trebi and Jet were different. With two independent genes, approximately 1/16 of the population should be susceptible, or 5 rows from an F_3 population of 89 rows. The presence of 3 susceptible rows was within the range expected for independence, giving a probability of 0.50–0.30 with the χ^2 test. However, with such low numbers the χ^2 test is of limited value. If the genes were on the same chromosome pair, it would require a crossover value of about 38 per cent to obtain a total of three susceptible rows in the above population since they entered the cross in the repulsion phase. Independence of the two genes was suggested in unpublished data involving the crosses Jet × Brachytic, C.I. 6572, and Trebi × Brachytic. There was evidence of close association between the Jet gene for resistance and the gene or genes for loose smut resistance in Brachytic. No such association was noted in the Trebi × Brachytic cross.

Inheritance of Resistance to Loose Smut in Hybrids with Dorsett

Dorsett was moderately resistant and selection X173-10-5-6-1 moderately susceptible under artificial inoculation, with 12.9 and 43.6 per cent infected plants, respectively. The reactions of the F_1 and F_2 generations of the cross X173-10-5-6-1 × Dorsett are shown in table 2. The infection in the F_1 generation was slightly less than the average for the susceptible variety and the infection of the F_2 exceeded it. This increased infection in the F_2 suggested that selection X173-10-5-6-1 possessed a gene or genes for intermediate smut reaction. These data were of importance as an aid in interpreting the distribution of the F_3 progeny. The infection in the F_1 generation was an estimate of the infection which occurred in plants heterozygous for the factors for resistance, or, by the same reasoning, the amount of protection the factors in the heterozygous condition gave the plants. The infection in the F_2 generation was an estimate of the amount of infection expected in F_3 rows from F_2 plants which were heterozygous for the resistant genes.

The distribution of parental and F_3 progeny rows is given in table 3 and shown graphically in figure 5. Transgressive segregation toward the susceptible side was apparent; several F_3 progeny rows were more susceptible than selection X173-10-5-6-1. Evidence, to be presented later, sug-

gested that some of the progeny rows were more resistant than Dorsett, although the increased resistance was not great.

Progenies from eleven noninoculated F_3 rows which had more than 63 per cent infection in the inoculated F_3 complement were tested in the F_4 generation. The results are given in table 5. The average infection for all tests of the eleven progenies was 66 per cent, compared with 36 per cent for selection X173-10-5-6-1. The infection level of the checks was lower than when the F_3 progenies were grown, which would account for the lower infection in the F_4 generation compared with the F_3 generation (Table 5). The data on F_4 tests of progenies from comparable F_3 rows which had less than 12 per cent infection in the F_3 are given in table 5. The average infection for all tests of the 14 rows was 6 per cent, compared with 12 per cent for Dorsett. These results suggested a segregation for slightly increased resistance.

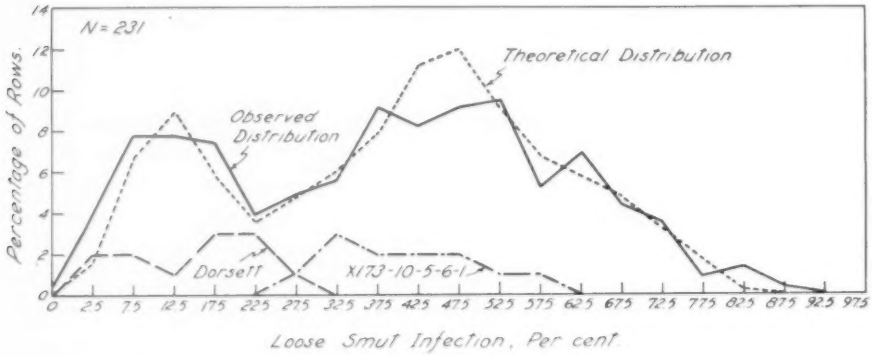

FIG. 5. X173-10-5-6-1 × Dorsett. Observed distribution of 231 F_3 lines in percentage infection classes compared with the theoretical distribution. Parental distributions are based on the number of tests rather than the percentage of rows in each infection class.

The bimodal curve given by the distribution of the F_3 lines suggested that resistance was controlled by relatively few genes. The hypothesis that each parent possessed a single gene for resistance satisfactorily explained the observed distribution. For convenience the genes contributed by Dorsett and selection X173-10-5-6-1 will be designated as D and X, respectively.

An attempt was made to assign infection values, consistent with known facts, to the F_3 rows representing the 9 different genotype combinations obtainable in a dihybrid ratio. On the basis of the parental reactions, the D and X genes permit approximately 13 per cent and 44 per cent infected plants, respectively, when in the homozygous condition. F_3 rows from F_2 plants homozygous for the parental gene combinations would be expected to have similar infection percentages. The F_3 lines from DDXX and DDXx plants should have as much or more resistance than Dorsett. This

was confirmed by F_4 tests. The lines homozygous for X and segregating for D, DdXX, would fall between the reactions of the two parents. The homozygous recessive lines, ddxx, would be completely susceptible.

The F_2 data (Table 2) indicated that F_3 rows from DdXx and ddXx plants should have an average infection of 48 and 64 per cent, respectively. As the D gene was more potent in conferring resistance than the X gene, lines homozygous recessive for X and segregating for the D gene, Ddxx, should fall somewhere between the values given above, namely 48 and 64 per cent. A value of 52.5 per cent was assigned as it met the requirements of the observed distribution. This value was substantiated by evidence in crosses of Dorsett with Trebi and Jet, which will be presented later.

The F_2 genotypes and the percentage of smut expected in F_3 rows are given below.

Ratio of F_2 genotypes		Mean percentage of smut in F_3 rows	Ratio of F_2 genotypes		Mean percentage of smut in F_3 rows
1	DDXX	7.5	2	Ddxx	52.5
2	DDXx	12.5	1	ddXX	40.0
2	DdXX	30.0	2	ddXx	62.5
4	DdXx	45.0	1	ddxx	72.5
1	DDxx	17.5			

The various genotypes were assigned values which were multiples of 2.5 per cent. Although the interactions of the genes could not be calculated accurately, the assigned mean values were consistent with what might reasonably be expected. In case of known reactions, such as parental types, the assigned values were within one standard deviation of the observed values.

Frequency distributions were calculated for the genotypes by using the means indicated and the standard errors derived from the formula $S = \sqrt{\frac{p\,q}{n}}$. While this probably underestimated the errors since no account is taken of environmental effects, it gave a distribution which comformed very well to that observed. The separate distributions for the genotypes are shown in table 6. The combined values of the separate distributions gave the number of rows expected for the various classes. The theoretical distribution is given in table 6 and shown graphically by the broken line in figure 5. The agreement between the observed and theoretical distributions was good; the χ^2 test gave a probability of 0.95 to 0.50.

Dorsett was crossed with the resistant varieties Trebi and Jet, each of which contained a gene for resistance. Table 3 gives the distribution of the F_3 progeny rows. Highly susceptible rows occurred in both crosses, indicating that the genes for resistance in the three varieties were different.

When two genes are present, a ratio of 15 resistant and segregating to 1 susceptible would be expected. In the cross with Trebi, 6 lines had more

than 65 per cent infection and would be classified as susceptible. From the population of 96 F_3 rows, 6 were expected. Twelve lines had between 35 and 60 per cent infection, comprising about 2/16 of the population. On the basis of previous crosses, lines segregating for the Trebi gene would have less than 30 per cent infection. Consequently, these 12 lines would probably be segregating for the D gene and recessive for the Trebi gene.

TABLE 5.—*Comparison of percentage smut infection of low and high F_3 lines with percentage infection in corresponding F_4 lines. X173-10-5-6-1 × Dorsett*

F_2 plant number	Infection in F_3 generation	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Pct.	*No.*	*Pct.*	*Pct.*
188	2	4	1	0–6
136	3	4	21	11–40
135	3	5	1	0–6
32	4	6	4	0–29
192	4	5	8	0–23
87	6	5	12	0–18
116	6	5	5	0–16
133	7	4	0	0
146	8	5	2	0–7
65	9	3	11	0–20
77	9	5	1	0–3
15	10	4	1	0–5
69	11	6	11	0–20
27	12	5	5	2–8
Av.	7	.....	6	.....
127	63	3	33	32–33
79	67	3	89	83–100
13	69	3	48	30–80
196	70	2	96	93–100
61	71	5	34	7–64
194	72	5	59	22–86
44	72	5	69	50–100
11	73	4	50	35–68
211	74	5	71	40–91
105	76	4	70	50–90
78	81	4	67	67–72
79	67	3	89	83–100
13	69	3	48	30–81
196	70	2	96	93–100
Av.	71	.....	66	.....
Dorsett	.....	16	12	0–37
X173-10-5-6-1	.....	16	36	22–60

The average infection of the 12 lines was 50 per cent which was in close agreement to the mean value of 52.5 per cent assigned to the lines segregating for D in the cross selection X173-10-5-6-1 × Dorsett.

While susceptible lines and lines with intermediate infection occurred in the cross Dorsett × Jet, there was a deficiency of these lines when compared with the number obtained in the cross Dorsett × Trebi (Table 3). In the latter cross there were 21 lines with 20 per cent or more infected plants,

TABLE 6.—*Theoretical distribution of F_3 rows of X173-10-5-6-1 × Dorsett for the genotypes indicated*

Infection classes	DDXX	DDXx	DDxx	DdXX	ddXX	DdXx	Ddxx	ddXx	ddxx	Rows expected	Rows observed	χ^2
Pct.										*No.*	*No.*	
0–5	3.04	0.65								3.69	10	3.8995
5–10[b]	8.35	6.67	0.61							15.63	18	
10–15	3.05	14.24	3.49							20.78	18	0.3719
15–20		6.67	6.24	0.79						13.70	17	0.7948
20–25		0.65	3.49	4.15						8.29	9	0.0603
25–30			0.61	9.50	0.53	0.14				10.78	11	0.0046
30–35				9.50	2.17	2.10				13.77	13	0.0435
35–40				4.15	4.52	8.77	0.38			17.82	21	0.5673
40–45				0.79	4.52	17.87	2.38			25.56	19	1.6834
45–50					2.17	17.87	6.72	0.31		27.07	21	1.3609
50–55					0.53	8.77	9.92	2.24		21.46	22	0.0135
55–60						2.10	6.72	6.86	0.08	15.76	12	0.8972
60–65						0.14	2.38	10.06	0.94	13.52	16	0.4548
65–70							0.38	6.86	3.50	10.74	10	0.0512
70–75								2.24	5.40	7.64	8	0.0170
75–80								0.31	3.50	3.81	2	
80–85									0.94	0.94	3	0.2836
85–90									0.08	0.08	1	
90–95												
95–100												
Total	14.44	28.88	14.44	28.88	14.44	57.76	28.88	28.88	14.44	231.04	231	10.5035

[a] Square of difference between number observed and number expected, divided by number expected.
[b] 5.1–10.0 per cent, inclusive.

compared with 13 lines in the Dorsett × Jet cross. If this difference was real, it would indicate a loose linkage between the D and J genes for resistance. Since the genes entered the cross in the repulsion phase, susceptible lines would result only from the union of crossover gametes, and lines with intermediate infection from the union of crossover gametes with noncrossovers. Consequently, a deficiency of these lines would be expected if the D and J genes were in the same linkage group. A crossover value of 34.6 per cent would be necessary to give the three susceptible rows that occurred.

While suggestive, the number of F_3 progeny tested was too small to establish definite linkage between the D and J genes. A crossover value of 34.6 per cent would give a distribution only slightly different from that expected from random assortment.

Association of Characters

In all of the crosses involving resistance *vs.* susceptibility, the parents differed in one or more morphological characters known to mark particular linkage groups. The markers used in this study have been found by several investigators to be controlled by single genes, and, with one exception, have been found to be inherited independently of each other. Daane (2) found the genes governing rough *vs.* smooth awns and long- *vs.* short-haired rachillas to be linked. A summary of these studies was reported by Robertson, Wiebe, and Immer (9), and therefore the individual references will not be cited here.

The variety Jet has a black lemma and pericarp, 2-rowed spikes, naked caryopsis, and full awns, and carries the allel for normal seedling color. In contrast, Colsess IV has a white lemma and pericarp, 6-rowed spikes, covered caryopsis, and hoods in place of awns, and carries the lethal seedling character, xantha, in the heterozygous condition. The genes conditioning these characters are located in linkage groups II, I, III, IV, and VI, respectively. Newal differs from Trebi in having smooth awns and long-haired rachillas. Both characters are markers for linkage group V. Dorsett is rough-awned, has a black lemma and pericarp and long-haired rachilla. Selection X173-10-5-6-1 has the allelmorph to each of these characters.

Classifications were made on F_2 plants and, in general, verified in F_3 lines. In the backcrosses, both the backcross plants and the progeny lines were classified. An attempt was made to verify previous reports as to the mode of inheritance of the genes, their relationships to each other and to the genes conditioning resistance to loose smut. Tests for goodness of fit and independence were made by means of χ^2. A P value greater than 0.05 was regarded as indicating satisfactory agreement.

The results of the χ^2 tests are summarized in table 7. With one exception, the segregation of the morphological characters fit monohybrid ratios, which is in agreement with published results. In the cross, Colsess IV × Jet, the segregation for non-6-rowed *vs.* 6-rowed spikes did not fit a 3:1

ratio because of an excess of 6-rowed segregates. However, in the backcross involving the same varieties, the normal 1:1 ratio was obtained for row

TABLE 7.—*Summary of χ^2 tests for mode of inheritance and relationship of characters in the various crosses*

Characters and crosses	Assumption	χ^2	D.F.	P
Newal × *Trebi* (N = 236)				
Rough *vs.* smooth awns (Rr)	3:1	0.08	1	0.80–0.70
Long- *vs.* short-haired rachilla (Ss)	3:1	1.19	1	0.30–0.20
Awns, rachilla hairs	9:3:3:1	13.70	3	< 0.01
Awns, rachilla hairs	Indep.	15.00	1	< 0.01
Smut infection, awns	Indep.	2.02	10	> 0.99
Smut infection, rachilla hairs	Indep.	5.85	10	0.90–0.80
F_1 (*Newal* × *Trebi*) × *Newal* (N = 57)				
Rough *vs.* smooth awns (Rr)	1:1	2.52	1	0.20–0.10
Smut infection, awns	Indep.	15.32	10	0.20–0.10
Colsess IV × *Jet* (N = 91)				
Black *vs.* white lemma and pericarp (Bb)	3:1	0.95	1	0.50–0.30
2-rowed *vs.* 6-rowed (Vv)	3:1	4.80	1	0.05–0.02
Hulled *vs.* naked (Nn)	3:1	0.05	1	0.90–0.80
Awns *vs.* hoods (Kk)	3:1	0.06	1	0.90–0.80
Lemma color, hull adherence	9:3:3:1	1.56	3	0.70–0.50
Lemma color, hoods	9:3:3:1	2.44	3	0.50–0.30
Hull adherence, hoods	9:3:3:1	0.53	3	0.95–0.90
Row number, hull adherence	Indep.	3.59	2	0.20–0.10
Row number, hoods	Indep.	5.85	2	0.10–0.05
Lemma color, row number	Indep.	5.79	2	0.10–0.05
Smut infection, lemma color	Indep.	5.42	6	0.50–0.30
Smut infection, row number	Indep.	1.63	6	0.95–0.90
Smut infection, hull adherence	Indep.	3.86	3	0.30–0.20
Smut infection, hoods	Indep.	9.63	6	0.20–0.10
Colsess IV × F_1 (*Colsess IV* × *Jet*) (N = 116)				
Black *vs.* white lemma and pericarp (Bb)	1:1	0.008	1	0.95–0.90
2-rowed *vs.* 6-rowed (Vv)	1:1	1.92	1	0.20–0.10
Awns *vs.* hoods (Kk)	1:1	0.42	1	0.70–0.50
Lemma color, row number	1:1:1:1	3.10	3	0.50–0.30
Lemma color, hoods	1:1:1:1	0.60	3	0.90–0.80
Row number, hoods	1:1:1:1	3.12	3	0.50–0.30
Xantha, lemma color	Indep.	1.20	1	0.30–0.20
Xantha, row number	Indep.	1.21	1	0.30–0.20
Smut infection, lemma color	Indep.	0.14	1	0.80–0.70
Smut infection, row number	Indep.	0.06	1	0.80–0.70
Smut infection, hoods	Indep.	0.14	1	0.80–0.70
Smut infection, Xantha	Indep.	0.00	1	> 0.99
X173-10-5-6-1 × *Dorsett* (N = 231)				
Rough *vs.* smooth awns (Rr)	3:1	0.64	1	0.50–0.30
Black *vs.* white lemma and pericarp (Bb)	3:1	0.24	1	0.70–0.50
Long- *vs.* short-haired rachilla (Ss)	3:1	0.64	1	0.50–0.30
Awns, lemma color	9:3:3:1	0.63	3	0.90–0.80
Awns, rachilla hairs	9:3:3:1	32.56	3	< 0.01
Awns, rachilla hairs	Indep.	28.30	1	< 0.01
Lemma color, rachilla hairs	9:3:3:1	1.13	3	0.80–0.70
Smut infection, awns	Indep.	6.79	10	0.80–0.70
Smut infection, lemma color	Indep.	6.98	10	0.80–0.70
Smut infection, rachilla hairs	Indep.	3.56	10	0.98–0.95

number. When selecting F_2 plants for inoculation, the only restriction imposed was that they produce sufficient seed for adequate F_3 tests. This

would tend to favor the 6-rowed types. Since there was no association between the genes conditioning smut reaction and row number (Table 7), the differential selection of 6-rowed segregates would not affect the final analysis of the genes involved in resistance.

The genes for rachilla hair length and awn barbing were found to be linked, with a percentage recombination of 29 ± 3.1 and 29 ± 3.6, in the crosses Newal × Trebi and selection X173-10-5-6-1 × Dorsett, respectively, when computed according to the method of Immer (3). These percentages are in agreement with those obtained by other investigators (9). All other morphological characters showed independent segregation.

Complete independence of the genes conditioning smut reaction and the various morphological characters was found in all crosses. Either the genes conditioning smut reaction were located in linkage groups other than those investigated or were so far removed from the marker genes that the linkage was not detected. Markers for 5 of the 7 linkage groups were present in the cross, Colsess IV × Jet, and the backcross, Colsess IV × F_1 (Colsess IV × Jet).

DISCUSSION

Four genes for resistance to loose smut of barley, *Ustilago nuda,* were found in a series of crosses between susceptible × resistant and resistant × resistant varieties. Resistance was found to be determined by the genetic constitution of the developing embryo rather than that of the surrounding floral tissue. The F_1 plants of the crosses Newal × Trebi and Colsess IV × Jet, with the susceptible variety as the female parent, were as resistant as the resistant parent. In this case, the heterozygous embryos were surrounded by susceptible floral tissue at the time of inoculation. The increased infection in the F_2 generation of the same crosses and their reciprocals showed that the fungus was able to become established in F_2 embryos which were surounded by maternal tissue having the F_1 complement of genes for resistance.

Reduced infection in the F_2 generation and in F_3 progeny lines from heterozygous plants suggested a slight amount of interference by the resistant maternal tissue in fungal penetration. This was noted by Livingston (5) in studies with loose smut of barley and also by Caldwell and Compton (1) in their studies on inheritance of resistance to loose smut in wheat. However, reduced germination of infected seed, selective mortality of infected plants, and any selectivity in the mortality of flowers after inoculation would give similar reductions. A definite reduction in plant numbers was noted in susceptible and segregating rows as compared with the resistant rows in the cross Newal × Trebi and to a less extent in other crosses. Although no measure was made of selectivity, the total mortality of inoculated flowers was fairly great.

The genetical analysis of the Trebi crosses agreed with the findings of Livingston (5), who reported a major gene for resistance in Trebi. This

gene was dominant in effect, the heterozygous plants being as resistant as Trebi, which occasionally had an infected plant. Robertson, Wiebe, and Shands (10) have assigned the symbol *Un* to this gene.

The high type of resistance in Jet was due to a single dominant gene. The heterozygous plants were as resistant as the parent. Jet was completely free of infection in all tests, both to the smut collection used in this study and to a composite of collections used in the breeding program. The occurrence of three susceptible F_3 lines in the cross Trebi × Jet indicated that the genes for resistance in Trebi and Jet were different. The population was too small to prove conclusively that these genes were in different linkage groups, but the data suggested independence.

The genetical analysis of the cross selection X173-10-5-6-1 × Dorsett was more difficult than for the previous crosses as more than one gene for resistance was present. Several F_3 lines were more susceptible than the susceptible parent. The presence of two independent genes for resistance, one contributed by each parent, satisfactorily explained the segregation in the F_3 rows. The genes in Dorsett and selection X173-10-5-6-1 differed from those in Trebi and Jet in that they permitted about 13 and 43 per cent of smut, respectively, when homozygous. The information available on the Dorsett and selection X173-10-5-6-1 genes indicated that they allowed 55 to 65 per cent smutted plants, respectively, when heterozygous. The individuals homozygous for both genes were slightly more resistant than Dorsett. The presence of the weak gene in selection X173-10-5-6-1 was substantiated further by the occurrence of 64 per cent infection in the F_2 generation of the cross Newal × X173-10-5-6-1, the parental varieties having an average infection of 70.5 and 43.6 per cent, respectively.

Segregation occurred in the crosses of Dorsett with Trebi and Jet. The Dorsett and Trebi genes were independent; the F_3 segregation gave the expected ratio of 15 resistant and segregating lines to one susceptible. Two-sixteenths of the lines had infection values ranging from 35 to 60 per cent, with an average infection of 50.0 per cent, which was in close agreement to the mean value of 52.5 per cent assigned to the lines segregating for the Dorsett gene in the cross selection X173-10-5-6-1 × Dorsett.

When the F_3 distribution of the crosses Dorsett × Jet and Dorsett × Trebi are compared, there is a noticeable deficiency of lines with more than 20 per cent infection in the former cross. Since the Trebi and Jet genes were similar in effect, parallel distributions would be expected if the genes were independent. A loose linkage between the Dorsett and Jet genes would account for this deficiency, as lines recessive for the Jet gene and either homozygous recessive or segregating for the Dorsett gene would result only from the recombination of genes from crossover gametes and the union of crossover with noncrossover gametes. Since the F_3 population was too small to establish the significance of this deficiency, it does not seem desir-

able to stress the possible linkage of the Dorsett and Jet genes until conclusive evidence is available.

Livingston (5) reported the presence of a weak gene in Missouri Early Beardless, which had an average infection of 53 per cent in his tests. However, this gene was ineffective against the smut collection used in the present study. In a number of tests with this collection, Missouri Early Beardless averaged 70 per cent infection and was classified as susceptible. Differences in varietal reaction showed that the weak gene in selection X173-10-5-6-1 was not the same as the one reported in Missouri Early Beardless.

While 4 genes for resistance were found, none occurred in combination. However, this might be expected as three of the varieties trace back to different places of origin, Trebi to Asiatic Turkey, Jet to Abyssinia, and Dorsett to Manchuria. It is expected that these genes would occur in other varieties originating in the same localities. Shands and Schaller (15) pointed out in their discussion that the intermediate type of resistance, as found in Dorsett, was common in the varieties from Manchuria, while those from Abyssinia had the highest proportion of varieties with the high type of resistance.

In keeping with the recommendations of Robertson, Wiebe, and Shands (10), the symbols Un_3, Un_4, and Un_5 are suggested for the genes in Jet, Dorsett, and selection X173-10-5-6-1, respectively. The symbols Un and Un_2 have been previously assigned to the Trebi gene and the weak gene in Missouri Early Beardless.

Independence of the genes conditioning loose smut reaction and the various morphological characters was found in all crosses. The Trebi gene for resistance was independent of those conditioning awn barbing and rachilla hair length, which are markers for linkage group V. Shands (14) reported a close linkage between the Trebi gene and the gene for stem rust resistance of Chevron, C.I. 1111, which is located in linkage group VII. A fairly close linkage was found between the Trebi gene and the gene for shortened internodes (Brbr), which is in group VII, in the cross Trebi × Brachytic, C.I. 6572 (unpublished data).

No association was found between the genes for resistance in selection X173-10-5-6-1 and Dorsett and those conditioning awn barbing, rachilla hair length, and lemma color.

Markers for six of the seven linkage groups were tested for their relationship to the Jet gene for smut resistance. Five linkage groups, I, II, III, IV, and VI, were involved in the cross Colsess IV × Jet and the backcross Colsess IV × F_1 (Colsess IV × Jet) (Table 7). The Trebi gene for smut resistance served as a marker for group VII in the cross Trebi × Jet. No evidence of linkage was detected with any of the six markers. If the Dorsett and Jet genes are linked as suggested earlier, independence of the Jet gene and the markers for linkage group V, awn barbing and rachilla hair length, can be assumed since the Dorestt gene was independent of

them. It is evident that the gene conditioning smut resistance in Jet was so far removed from the marker characters used in this investigation that linkage could not be detected.

SUMMARY

Evidence was presented which demonstrated the existence of four genes for resistance to loose smut of barley, three of which have not been reported previously. Each of the four varieties analyzed possessed a single gene which conferred its own degree of resistance. The genes in Trebi and Jet gave the highest type of resistance and were dominant in effect. The Dorsett and selection X173-10-5-6-1 genes allowed about 13 and 43 per cent of smutted plants when homozygous, and 55 and 65 per cent when heterozygous, respectively. The symbols Un_3, Un_4, and Un_5 were suggested for the genes in Jet and Dorsett and selection X173-10-5-6-1, respectively.

The Trebi gene was independent of the Jet and Dorsett genes. The Dorsett gene and the weak gene in selection X173-10-5-6-1 were also independent. Linkage between the Dorsett and Jet gene was suggested, but the evidence was not conclusive.

No associations were found between the genes for resistance and marker genes for the various linkage groups.

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PRODUCTION AND DISCHARGE OF BASIDIOSPORES BY PELLICULARIA FILAMENTOSA (PAT.) ROGERS ON HEVEA RUBBER[1]

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(Accepted for publication July 5, 1949)

A study of the production and discharge of basidiospores by *Pellicularia filamentosa* (Pat.) Rogers was made in connection with investigations on the Pellicularia leaf spot[3,4] of Hevea rubber trees, *Hevea brasiliensis* (H.B.K.) Muell.-Arg. and other species, in eastern Perú. In December 1947 an intensive search was made for the perfect stage of the fungus on infected leaves collected and examined during daylight hours. Although abundant hymenia were found, they had almost no basidia with either sterigmata or sterigmata and basidiospores, though some collapsed basidia were seen. As the fungus spreads rapidly under favorable conditions, it was assumed that it must sporulate heavily and frequently, if not in the daytime then perhaps at night. This paper describes the experiments and observations made during a study of the sporulation phenomena.

METHODS AND MATERIALS

The greater part of the work dealt with the behavior of the fungus on naturally-infected leaves bearing large lesions with an extensive hymenium, which appeared as a silvery bloom within the diseased area on the dorsal side of the leaf. The methods used for studying basidiospore discharge were modified after those employed by Keitt and Jones[5] and Keitt *et al.*[6]

A suitable portion of leaf, at least 3×5 cm., was removed and put into a moist chamber, which was usually a Petri dish cover. The ventral side of the leaf piece was pressed against a wet filter paper in the inside top of the chamber, without touching or wetting the hymenial surface. The

[1] Grateful acknowledgment is made to Dr. Vincent E. Iverson, Technical Adviser to the Horticulture Department, Estación Central de Colonización en Tingo María, for valuable suggestions during the preparation of this paper, and to the Directors of the Estación for the facilities extended.

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chambers were arranged on a base covered with moist filter paper, so that each leaf piece was over a countersunk wooden drawslide, just wide enough to accommodate a glass microscope slide and long enough to permit the insertion and removal of slides. Thus, successive slides were exposed beneath the same leaf surface, without seriously disturbing the air within the chamber, or the chamber itself. The slides were coated lightly with glycerin jelly.

High humidity was maintained in the chambers; each was opened once daily for a few minutes to collapse the often abundant, sterile, aerial mycelium, and to permit moistening of the filter papers. The chambers were then replaced in their original positions.

Spore counts were made as follows. Cotton blue in lacto-phenol was placed on the slide and covered with a 50×24 mm. coverglass. Fifty well-distributed fields per slide were counted or estimated, using a mechanical stage. The results are expressed as the average number of basidiospores per sq. cm. per time interval.

Unless otherwise noted, the trials were made in a white-walled room with two large uncurtained windows on the east and west sides respectively, so that the chambers were in strong diffuse natural light during the day and normal darkness at night.

The fungus was also grown in pure culture on soil agar, where it produced a fertile hymenium within a few days on the sides of the culture vessel, which was usually a half-pint milk bottle. The agar was prepared as follows: A few grams of moist soil with a high content of organic matter and 3 or 4 wheat seeds were placed in each vessel, steamed for 30 min., and left overnight. The following day, water agar was added to a total depth of 1 cm. and the vessels were plugged, autoclaved for 45 min. at 15 lb. pressure, cooled in a horizontal position, inoculated, and incubated in a horizontal position at room temperature.

Also, hymenia were produced by transferring a 1×2 cm. portion of an active plate culture, preferably on deep soil agar, to a sterile Petri dish containing 1 or 2 cc. of water agar along one side of the plate to help maintain high humidity. The block was placed at some distance from it. Fertile hymenia were produced within 24 to 48 hr. on the glass and could be studied directly without being disturbed.

EXPERIMENTAL RESULTS

First to be studied were the daily fluctuations in basidiospore discharge under natural light conditions. The data given in table 1 were obtained from two trials made 9 months apart and are in agreement with other simpler preliminary trials. Data on basidiospore discharge are presented by 6-hr. intervals, since detailed data for a 4-day period appear in table 2. It may be seen that the bulk of the spores were discharged during the

night, from 6 P.M. to 6 A.M., with a much lower rate of discharge during the daytime, especially on sunny days. All the hymenia studied gave relatively heavy discharges at night throughout the trial period, but many gave no discharge during a period of 6 to 12 hr. in the daytime.

The ability of the fungus to continue growth and active spore production on detached leaf pieces, over a period of several days, suggests that lesions on favorably disposed fallen leaves may continue to produce abundant inoculum for some time. During the initial search for the perfect stage, numerous well-developed active hymenia were found on such leaves.

The second phase of experimentation was a preliminary inquiry into the factors governing sporulation. In a 3-part experiment in February 1949, the material was subjected to (a) natural light, (b) prolonged light,

TABLE 1.—*Basidiospore discharge[a] under natural light by Pellicularia filamentosa on infected leaves of Hevea brasiliensis*

Interval (in hours)	Trial A[b] day number					Trial B[c] day number			
	1	2	3	4	5	1	2	3	4
6 P.M. to midnight	207	1248	7953	8767	2989	675	15271	13078	13123
Midnight to 6 A.M.	179	8	3130	1855	535	2514	8338	9625	6713
6 A.M. to noon	T[d]	77	10	17	2[e]	108	143	305	254[e]
Noon to 6 P.M.	T	35	T	0		569	757	369	

[a] Basidiospore discharge expressed as average number of spores per cm.^{2} based on counts of 50 fields per slide.

[b] Begun April 13, 1948; one leaf only; bright sunny days.

[c] Begun January 12, 1949. One leaf only first day, average of 6 leaves thereafter; cloudy nearly all day every day.

[d] Trace, less than 1 spore per cm.^{2}

[e] Ended 8 A.M.

and (c) prolonged darkness, with the resumption of alternating light and dark periods in each (b) and (c) after 42 hr., which period had embraced two night and day cycles. Six leaf pieces were used in each part of the trial. Three pieces were cut from each of 6 leaves bearing extensive hymenia, and one piece was used in each part of the experiment, so that each "number 1" leaf piece had a common origin. Fortunately, all 6 leaves had fertile hymenia and it seems safe to assume that the principal variations among the treatments were due to environment and not to differences in the test materials.

The controlled light was furnished by a 100-watt Mazda lamp in a reflector, at 18 in. from the chambers. Darkness was obtained in a windowless closet.

No critical temperatures were encountered within the range of 21.5° to 28° C.; the temperature usually varied between 23° and 25° C.

The data from this experiment are presented in table 2. Basidiospore discharge under natural light conditions had a similar distribution to that

in the trials reported in table 1, with the heavy sporulation rate at night and a very low sporulation rate during the day, over a period of four night-and-day cycles. Under prolonged light, sporulation was retarded for about 6 hr. beyond the end of normal daylight, after which substantial sporulation occurred in two distinct cycles of 12 and 15 hr. each, separated by a 9-hr. interval of low sporulation. In prolonged darkness, heavy discharge occurred during the two normal night periods, but was low during the periods corresponding to daytime.

TABLE 2.—*Effect of various light conditions on basidiospore discharge*[a] *by Pellicularia filamentosa on infected leaves of Hevea brasiliensis*

Day	Interval, in 3-hr. periods	Light conditions[b]		
		Natural	Controlled	
			Prolonged light	Prolonged darkness
1	6 P.M.	617	2	471
	9 P.M.	3645	16	4657
	Midnight	4376	416	3474
	3 A.M.	1052	370	658
	6 A.M.	66	671	23
	9 A.M.	0	920	50
	Noon	0	195	135
	3 P.M.	0	0	219
2	6 P.M.	2771	132	364
	9 P.M.	8018	1968	2318
	Midnight	4822	1367	5287
	3 A.M.	2262	994	4876
	6 A.M.	164	1150	2634
	9 A.M.	2	1024	938
	Noon	0	1798	163
	3 P.M.	2	5007	62
3	6 P.M.	4494	4388	730
	9 P.M.	10999	2008	5128
	Midnight			
	3 A.M.	1761	T[c]	7992
	6 A.M.	118	0	5586
	9 A.M.	0	0	4834
	Noon	0	0	2686
	3 P.M.	8	381	52
4	6 P.M.	3016	3663	5
	9 P.M.	5150	4990	17
	Midnight	2306	3836	305
	3 A.M.	719	417	2171
	6 A.M.	71	3	3850
	9 A.M.	0	End	3059
	Noon	End		1639
	3 P.M.			58
5	6 P.M.			0
	9 P.M.			End

[a] Basidiospore discharge expressed as average number of spores per cm.^{2}, based on counts of 50 fields per slide, using 6 leaf pieces in each series.

[b] Natural light indicates strong diffuse daylight and darkness in normal cycle; controlled light provided by a 100-watt Mazda lamp; controlled darkness obtained in a windowless closet. Boldface type indicates intervals of darkness. Under natural conditions, darkness prevails from about 7 P.M. to 6 A.M.

[c] Trace, less than 1 spore per cm.^{2}

When the controlled light series was returned to a sequence of typical light and dark periods, sporulation was similar to that found under natural light conditions, although the hymenia originally in prolonged darkness did not seem to readjust themselves as rapidly as those under prolonged light.

The third phase of the work dealt with a study of sporulation by the fungus when grown *in vitro* on soil agar. The slides, which were exposed beneath hymenia growing in half-pint milk bottles, were held by a wooden snap-clothespin, one arm of which was shortened so that the other could be inserted into a No. 8 rubber stopper, which replaced the cotton plug. Although an aseptic technique was not used, contamination offered no difficulties over a period of a few days.

Representative data are presented in table 3, indicating that sporulation in pure culture is similar to that encountered on naturally-infected leaves.

TABLE 3.—*Basidiospore discharge*[a] *by Pellicularia filamentosa in vitro and under natural light*

Interval, in hours	Day number				
	1	2	3	4	5
8 P.M. to midnight	123	267	1732	2343	2309
Midnight to 6 A.M.	404	351	1271	1293	5779
6 A.M. to noon	4	T[b]	2	0	32
Noon to 6 P.M.	0	1	0	0	0

[a] Basidiospore discharge expressed as average number of spores per cm.^{2} based on counts of 50 fields per slide, for each of 5 replicates. Experiment begun on March 4, 1949.

[b] Trace, less than 1 spore per cm.^{2}

Using the Petri dish technique for producing hymenia *in vitro,* it was possible to observe microscopically the changes that took place in the fungus. In a typical example, the following microscopic observations were made under natural light conditions, observing the same area throughout. The fungus was illuminated for about 1 min. at each observation.

3 A.M. The basidia appeared as dense, almost spherical bodies grouped on short lateral hyphae.

5:30 A.M. A few basidia had short sterigmata.

9:10 P.M. There was abundant spore production, with typical mature basidiospores borne on long sterigmata. No actual discharge was seen, although occasional violent action was noted in some denser parts of the mycelium.

11 P.M. Similar to 9:10 P.M.

5:30 A.M. There were almost no full-grown spores *in situ,* although some sterigmata still bore small, immature spores. Clearing and collapse of the spent basidia were beginning.

10 A.M. The spent basidia were distinctly collapsed and the immature basidia were still without sterigmata.

DISCUSSION

Basidiospore production and discharge in the *Hevea* strain of *Pellicularia filamentosa* appear to be a periodic function which may be strongly influenced by the presence or absence of light, at least over a period of a few days. Under natural light, the heaviest spore production and discharge occurred at night; sporulation in the daytime was low or absent. Since sporulation was sharply reduced even by diffuse daylight, it might be assumed that under the strong light found in the field daytime sporulation is negligible except in heavily shaded areas. No critical temperatures were encountered during the experiments. High humidity was favorable for the organism; no study was made on the effect of low humidities.

This fungus seems to be well adapted for persistence and for parasitizing the host, within its environment. The period of heavy sporulation coincides with the period of lower air temperature and increased humidity, commonly accompanied by a heavy dew formation that moistens the leaves of the host and provides conditions favorable for both the production and germination of basidiospores, since the temperature seldom falls below 16° C. The frequency with which the heaviest discharge occurs between 6 P.M. and midnight, or the first 6 hr. of darkness, appears to be more than a coincidence, since it would permit a long infection period for a maximum amount of inoculum.

The dew persists until after dawn in the Peruvian rubber-growing areas, and ground fogs frequently retard drying, even during the dry season. During the rainy season, night and early morning rains, and the prevailing high humidity, give conditions leading to severe disease development. Some new infections may always be found even in the dry season, when daytime temperatures are relatively high and the humidity is low, and there is little rainfall.

SUMMARY

Sporulation by the *Hevea* strain of *Pellicularia filamentosa* under natural light, both on infected leaves and *in vitro*, appears to be a periodic function, with the heaviest rate of basidiospore discharge occurring during the night and only a low to negligible rate of sporulation during the daytime.

Preliminary results suggest that periodic sporulation may be strongly influenced by manipulation of the light and dark periods to which the fungus is exposed.

The *Hevea* strain of *P. filamentosa* appears to be well adapted for persistence and for parasitizing the host within its environment.

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PROGRESS IN THE STUDY OF DWARF BUNT OF WINTER WHEAT IN THE PACIFIC NORTHWEST[1]

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Dwarf bunt (*Tilletia caries* (DC) Tul.) of winter wheat is widely prevalent in the Pacific Northwest[3] and has a more limited distribution in other regions (2, 11, 14). Because of certain characteristics (1, 5, 7, 8, 10, 15), some of which distinguish it from common bunt, dwarf bunt presents unique problems of investigation and control. Consequently, little is known about the nature of this disease and the biology of its causal fungus. In 1942 a series of studies was begun to elucidate some of the factors relating to the behavior and control of dwarf bunt. This paper constitutes a report on the progress of these studies.

MATERIALS AND METHODS

The scope of these investigations extended from field observations on factors relating to the occurrence of dwarf bunt to experimentation on the biology of the causal organism. Annual observations were made to determine the prevalence and severity of the disease under varying weather and soil conditions, and the tendency toward cyclic occurrence of epiphytotics. Observations were made on reactions of varieties in commercial and experimental plantings in different localities, both from the standpoint of resistance to dwarf bunt and that of the characteristics of diseased plants.

Nine collections of dwarf bunt were used in studies on physiologic specialization, chlamydospore germination, and nuclear behavior. Five of these collections were from Washington, two from Idaho, and one each from Montana and Utah. The spores of all collections were treated according to the method described previously (6), and inoculations were made by dipping the wheat kernels in a suspension of treated spores, after which the seed was dried before planting. All germination tests in the laboratory were made on 2 per cent water agar at approximately 8° C. Studies on nuclear behavior were made with material that was stained with Heiden-

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Idaho, Montana, Utah, and Washington Agricultural Experiment Stations. Published as Scientific Paper No. 830, Washington Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

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[3] The Pacific Northwest, as used in this paper, refers to the winter wheat producing regions of Idaho, Montana, Oregon, Utah, and Washington.

hain's iron-alum haematoxylin. Other materials and methods will be described with the presentation of results.

FIELD OBSERVATIONS AND EXPERIMENTAL RESULTS

Seed Inoculation

Failure to obtain infection from seed-borne chlamydospores of dwarf bunt is one of the major handicaps in the investigation of this disease. Within the last 15 years many routine inoculations have been made with dwarf bunt spores at experiment stations in the Pacific Northwest. Almost without exception the results from these tests have been negative. One exception worthy of note was recorded at Logan, Utah, in 1946. Seed of a susceptible variety (Turkey 926), naturally contaminated with dwarf bunt spores, was planted in irrigated soil and produced a crop with 25 per cent dwarf bunt. No dwarf bunt occurred in several other susceptible varieties included in this test and grown from noncontaminated seed. In this instance, therefore, an appreciable amount of infection was obtained from seed-borne inoculum. On the whole, however, experimental evidence and experience prove that this method of making dwarf bunt tests is not reliable.

The tests referred to above were made with chlamydospores as collected from the field. Additional tests were made with dwarf bunt spores which were given special treatment, including presoaking at low temperature, which is known to induce germination (6). For one test with spring wheat at Bozeman, Montana, in 1943, smutted heads of wheat were stored dry in the laboratory in the fall of 1942. In February 1943 some of this material was placed outside in the snow and allowed to remain until May, and the remainder was kept in the laboratory. Seed of two susceptible spring wheat varieties (Ulka, C.I. 11478, and Ceres, C.I. 6900) was inoculated with dwarf bunt spores of both treatments. No infection was obtained in this test.

In a test at Pullman, Washington, seed of several susceptible winter wheat varieties was inoculated with spores of 9 collections of dwarf bunt. These spores had been presoaked in water in a refrigerator for six months (6). The inoculated seed was planted in the field in October. No infection was obtained in any of the varieties.

Since dwarf bunt can seldom be reproduced by seed inoculation with chlamydospores, most of the information on its behavior has been obtained from field observations. During the course of this work special emphasis has been placed upon observations and studies of factors pertaining to the natural occurrence of the disease in commercial wheat fields. These have revealed certain phenomena relative to the incidence of dwarf bunt which may or may not have a bearing on final solution of the problem.

Perennial Nature of Soil Infestation

Dwarf bunt, being primarily a problem of soil infestation, cannot be controlled by seed treatment. It can be controlled by growing resistant varieties. Crop rotation has been considered as another possible means of control. However, successful control of any disease by this method depends upon how long the infestation from any one crop persists in the soil. That dwarf bunt persists for at least two seasons is suggested by the fact that there seems to be no correlation between the severity of the disease in one year and that in the succeeding year. Numerous observations in commercial fields have shown that heavy infestations frequently follow years of light infestation and vice versa.

Alternating years of light and heavy incidence of dwarf bunt appeared to be the rule in the infested areas of Montana. In the ten-year period, 1938–1948, records show that in the Gallatin Valley near Bozeman there was severe incidence of dwarf bunt in the even-numbered years, in contrast to relatively light incidence in the odd-numbered years. Fields with 75 per cent dwarf bunt were common in this area in the heavy-incidence years during the first half of this period, before resistant varieties became generally grown. By contrast, in the light-incidence years dwarf bunt occurred only in traces or small percentages. The only exception to this was in 1943 when some fields had spots up to an acre in size with as much as 50 per cent dwarf bunt.

The pattern of alternating years of light and heavy incidence was likewise typical of the Flathead Valley near Kalispell, Montana. There, however, the light and heavy infestations were in even and odd years, respectively, which is in the reverse order of that in the Gallatin Valley. The alternating pattern in the Flathead Valley was broken in 1946, when dwarf-bunt incidence was light.

In other areas, alternating years of light and heavy incidence of dwarf bunt are not the rule. Nevertheless, recorded observations in all areas of infestation show a definite lack of correlation between the amounts of dwarf bunt in successive years. For example, in southern Idaho and northern Utah the incidence of dwarf bunt was unusually severe in 1935; in many commercial fields estimated percentages of dwarf bunt were above 50 per cent and occasional ones had more than 75 per cent. Although this devastating incidence followed and was in turn followed by years (1934, 1936) of mild dwarf bunt, an alternating cycle was not evident in other years. In this area it has been observed that severe dwarf-bunt incidence usually follows early fall emergence of wheat, although at least one year (1946) was an exception to this rule.

There is some evidence that infective inoculum of dwarf bunt persists in the soil much longer than 2 years. For example, in old centers of infestation where resistant varieties have been grown for 7 to 10 years, thus

reducing to a minimum the amount of reinfestation, high incidence of dwarf bunt has been obtained in experimental plantings of susceptible varieties. In a commercial field in Utah which was at one time heavily infested and had been planted to resistant varieties for 7 years, as much as 65 per cent dwarf bunt occurred in the susceptible varieties of an experimental planting in 1946. Similar observations were made in Montana where two heavily infested fields in 1942 were grown to the resistant variety Wasatch in all succeeding crop years. The susceptible varieties in an experimental planting in these fields in 1948 averaged above 50 per cent dwarf bunt. The area surrounding these fields had likewise been relatively free of dwarf bunt for 4 years, owing to the use of resistant varieties. These observations suggest, therefore, that dwarf-bunt infestation of the soil may persist for at least 4 and possibly as long as 7 years.

Further evidence of the perennial survival of dwarf-bunt inoculum in the soil was obtained from an experiment[4] in which contaminated soil was protected from recontamination by wind-borne spores. Spore-proof boxes were used to cover plots that had been inoculated with dwarf bunt in the form of smutted heads of wheat, smut balls, and powdered spores. Each fall for three years the boxes were removed and the susceptible variety Utah Kanred was planted. Up to 20 per cent dwarf bunt occurred in these plots and there was no difference in the amount of infection where different kinds of inoculum were used. No dwarf bunt developed in the check plots, thus indicating that the boxes effectively protected the soil from contamination by wind-borne spores. This is additional evidence that dwarf bunt can persist in the soil for several years and for this reason it cannot be controlled by crop rotation.

Conditions Favoring Development of Dwarf Bunt

Little is known about the specific environmental requirements for the development of dwarf bunt. The nature of the disease and its causal agent has made it practically impossible to determine these requirements experimentally. Field observations during the last 15 years, which suggest certain conditions under which dwarf bunt develops most severely, are presented primarily as a basis for future study and observations.

Dwarf bunt occurs under a wide range of conditions in the Pacific Northwest. Early records indicate that it was most severe in low-rainfall, light-soil regions where the Turkey (Hard Red Winter) wheats predominate. More recently, however, it has reached destructive proportions in areas of higher rainfall and heavy soils where soft white and soft red winter wheats predominate. Furthermore, it occurs in irrigated winter wheat in the Gallatin Valley of Montana. Although there is no evidence that dwarf bunt has infested the irrigated soils of Cache Valley in Utah, it has

[4] Planned and conducted by H. A. Rodenhiser in cooperation with R. W. Woodward.

been produced experimentally under irrigation at Logan. In this experiment irrigated plots were inoculated with 2 gm. of spores per square foot 5 days before seeding a nursery of 25 varieties. A duplicate nursery was seeded in naturally infested soil under dry-farm conditions. The percentages of dwarf bunt on 7 susceptible varieties in these two nurseries are presented in table 1. It is apparent from these data that dwarf bunt develops at least as well under irrigation as under dry-farm conditions. Thus, from the above observations and experimental data it is evident that dwarf bunt is widely adapted in its soil and moisture requirements for good development.

Although there is no indication that soil type is a critical factor in dwarf-bunt development, there is some evidence that soil condition has a pronounced influence in this respect. Recorded observations show that this

TABLE 1.—*The percentages of dwarf bunt produced in susceptible wheat varieties in artificially inoculated irrigated plots and in naturally contaminated dry-farm plots at Logan, Utah, in 1946*

Variety	C.I. Number	Percentages of dwarf bunt in	
		Irrigated plots	Dry-farm plots
Hybrid 128	4512	90	63
Ridit	6703	5	2
Yogo	8033	65	70
Rio	10061	75	37
Brevon	11912	63	6
Elgin	11755	80	75
Minturki	6155	17	5

disease has a tendency to occur with variable severity in different parts of the same field. Localized spots of severe infestation varying from a few square feet to an acre have been observed in all areas of infestation. This is particularly true in regions of new infestation. More frequently than not, infection centers of this type occur where the soil becomes excessively compact; for example, dwarf bunt has repeatedly been observed to be more severe along certain slopes and ravines and at turning corners, and in the drill rows over which the wheels pass in the seeding operation.

The effect of packing the soil on the development of dwarf bunt was tested experimentally at Bozeman, Montana. Seed of the dwarf-bunt-susceptible variety Newturk was sown in a commercial field known to be heavily infested with dwarf-bunt inoculum. The seed was planted in rows 20 ft. long and replicated four times. In half of the rows the soil was compacted over the seed by tamping, the other rows being left as checks. The average percentage of dwarf bunt in the tamped rows was 58 while in the check rows it was 24, thus indicating that a compact condition of the soil favors dwarf-bunt development.

Degree of Dwarfing

Dwarf bunt reduces the height of infected plants to a striking degree. The percentage of reduction in height was determined on six different varieties by measuring 100 culms each of smutted and nonsmutted plants in commercial wheat fields. Three varieties near Bozeman, Montana, and three other varieties in experimental plots near Logan, Utah, were measured. The data are presented table 2.

The average height of smutted plants in four fields of Turkey wheat ranged from 8.44 to 15.62 in. compared with 24.80 to 39.86 in. for nonsmutted plants. This represents a reduction in height ranging from 58 to 66 per cent, an average of about 63 per cent. The average reduction in plant height in three fields of Turkey in 1942 was 66 per cent. Smutted plants in one field of Cache were reduced in height by 56 per cent in 1943 (Table 2), and 59 per cent in 1942. A similar degree of reduction in

TABLE 2.—*Degree of height reduction caused by dwarf bunt in six varieties of winter wheat*

Field No.	Variety	Year	Average height in inches		Percentage reduction in height
			Smutted	Healthy	
1	Turkey	1943	13.74	39.86	66
2	Turkey	1943	9.92	27.12	63
3	Turkey	1943	8.84	24.80	66
4	Turkey	1944	15.62	37.08	58
5	Cache	1944	17.76	40.06	56
6	Tenmarq	1944	13.73	37.23	63
7	Tenmarq	1946	10.72	27.22	60
8	Triplet	1948	11.61	23.73	51
9	Utah Kanred	1948	11.27	26.19	57
10	Minhardi	1948	11.59	28.25	59

height was found in Utah Kanred and Minhardi. Height reduction in the variety Tenmarq was intermediate between that of Turkey and Cache (Table 2). Smutted Triplet plants were about one-half the height of healthy plants of that variety. In Turkey and Tenmarq the infected plants were about one-third the height of healthy plants. In the other varieties studied the reduction was somewhat less, the height of smutted plants being between one-half and one-third that of healthy plants. Thus it appears that degree of height reduction caused by dwarf bunt depends upon the variety infected.

The slight difference in degree of stunting in Turkey and Cache may be due to the tendency toward partial smutting in the latter variety. In Turkey, partially smutted plants and heads are a rarity while in Cache they occur frequently. Invariably, the smutted culms of a partially smutted plant of any variety are taller than those of a completely smutted

plant. Likewise, partially smutted heads are higher than completely smutted heads of the same plant. In a commercial field of Pawnee near Waterville, Washington, which had 15 per cent dwarf bunt, the partially smutted heads were one-third to one-half as high as the nonsmutted heads. In contrast, however, there was no partial smutting in Triplet, and infected plants of this variety exhibited the least degree of dwarfing.

Physiologic Specialization

Since dwarf bunt is primarily a problem of soil contamination, the most effective method of control is to grow resistant varieties. Thus far, this method has been highly successful throughout the dwarf-bunt-infested area. However, its continued success will depend largely upon whether there are distinct pathogenic entities which cause dwarf bunt. Determination of this fact has been delayed because the dwarf-bunt disease cannot readily be reproduced by the usual process of seed inoculation with chlamydospores.

Physiologic races of the bunt fungi usually differ in one or more of several characters, the most important being pathogenicity. The dwarf bunt race differs from other races in at least 8 characters, including pathogenicity (5, 6, 7, 15). The possibility that pathogenic races of the dwarf-bunt fungus exist is suggested by the fact that distinct entities based on other criteria are readily recognized. For example, among nine collections studied, distinct differences were noted in spore germination, spore size and markings, nuclear behavior, number of sporidia produced, and effect on host morphology.

In a preliminary report (5) on dwarf bunt, it was shown that the chlamydospores were nonviable under laboratory conditions, germination being limited to certain hyaline, apparently haploid spores. Later (6) it was found that germination of the regular diploid spores could be induced by prolonged soaking in water at a low temperature. It appeared that this information might facilitate further studies on the dwarf bunt, especially in studies where spore germination is essential, as in pathogenicity. Attempts were made, therefore, to determine the pathogenicity of dwarf-bunt collections from different areas of infestation in the Pacific Northwest. The spores were treated by presoaking in water in the refrigerator for six months. These spores were then used to inoculate the seed of the winter wheat host testers for common bunt races (11). Duplicate nurseries were grown at Pullman, Washington, and Bozeman, Montana. The results were negative, no smut being produced on any of the varieties, despite the fact that at least some germination of spores occurred in all of the collections under laboratory conditions. The incubation period required for germination in the laboratory, however, was 4 to 6 weeks. Assuming that a similar incubation period is required under field conditions no infection could

be expected, since wheat seedlings probably develop beyond the susceptible stage within that time.

Among the nine collections of dwarf bunt studied, spore germination ranged from a trace in a collection from Bozeman, Montana, to as high as 50 per cent in a collection from Troy, Idaho. The presoak treatment (6) was essential to germination of the spores of all collections except the one from Troy, Idaho. Spores of this collection, without presoaking, germinated up to 50 per cent after incubating 4 weeks. Presoaking the spores of this collection increased the speed of germination slightly but not the percentage of germination. In general, presoaking for periods longer than 6 months did not increase either speed or percentage of germination in any of the collections, and periods longer than 12 months were detrimental to germination. In the collection from Troy, Idaho, no germination occurred in spores that were presoaked 21 months while this same length of treatment did not greatly impair germination in a collection from Waterville,

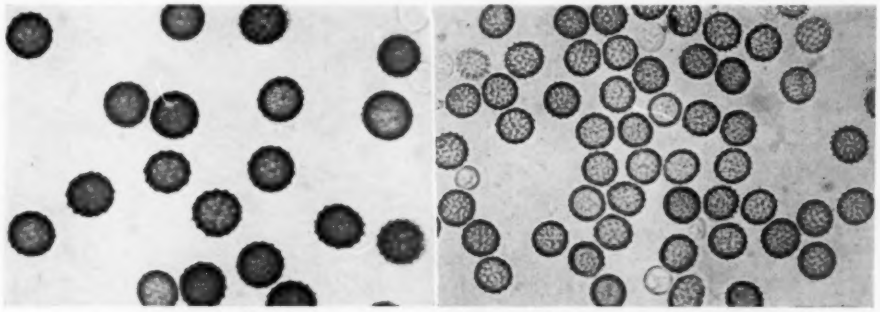

FIG. 1. Photomicrograph illustration of difference in size of chlamydospores of two collections of dwarf bunt. Left, collection from Bozeman, Montana; average diameter, about 20 microns. Right, collection from Troy, Idaho; average diameter, about 18 microns.

Washington. Thus, on the basis of difference in spore germinability there is evidence that dwarf bunt is caused by distinct physiologic entities of *Tilletia caries*.

Chlamydospore characteristics may also be used to differentiate between entities of the dwarf bunt. Prominence of the spore wall reticulations is one of the features which distinguish dwarf bunt from the common bunt races of *Tilletia caries*. However, there are differences in the degree of prominence of reticulations in various collections of dwarf bunt. For example, the spores of the collection from Waterville, Washington, are less prominently reticulate than those of the collection from Bozeman, Montana. Furthermore, the spores of the latter are larger and darker in color than those of the former collection. Spores of the collection from Bozeman are also larger than those of the collection from Troy, Idaho (Fig. 1).

Further indication of specialization in dwarf bunt is the difference in number of primary sporidia produced by the spores of collections from

different areas. There was also a difference in the tendency for promycelial branching. The number of sporidia on 11 germinating spores of the collection from Utah ranged from 28 to 66. Branching of the promycelia was common in this collection and the number of sporidia on the different branches varied. One promycelium had three branches with 13, 17, and 20, or a total of 50 sporidia. Only one spore had as many as 66 sporidia. The promycelium of this spore had two branches, one with 36 and the other with 30 sporidia. The average number of sporidia on these 11 promycelia was 45. The number of sporidia on the promycelia of 50 germinating spores of the collection from Troy, Idaho, ranged from 9 to 20, more than half of them ranging between 13 and 16. The average number of sporidia

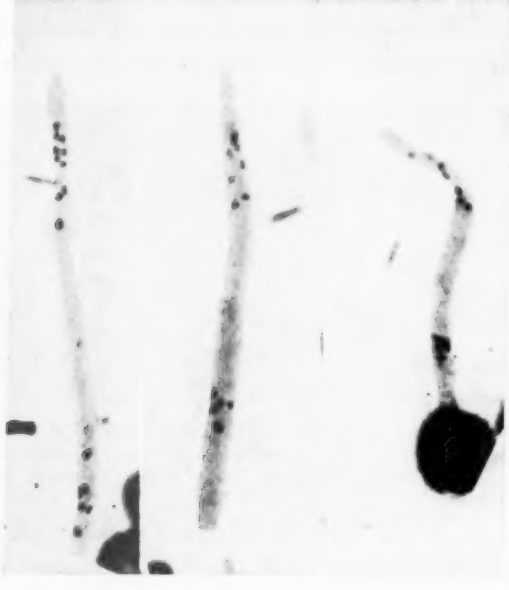

FIG. 2. Photomicrograph showing the aggregation of nuclei into two groups in the promycelia of three germinating chlamydospores of one collection of dwarf bunt.

for these 50 promycelia was 14.8. Branching of the promycelia in this collection was the exception rather than the rule.

Nuclear behavior in different collections of dwarf bunt suggests specialization in this respect also. In general, the nuclear phenomena in dwarf bunt resemble those of common bunt. The promycelium is multinucleate and one nucleus passes into each primary sporidium. Fusion of the primary sporidia is followed by the migration of the nucleus from one sporidium into the other, thus initiating the dikaryophase. Secondary sporidia, which are the product of nonfused primary sporidia, usually are uninucleate but occasionally may be binucleate.

A notable feature of the nuclei in the promycelia of germinating dwarf bunt spores was their tendency to be aggregated into two groups, one

group near the base and the other near the tip (Fig. 2). In the collection from High Prairie, Washington, the nuclei were aggregated into two groups in 45 out of 51 promycelia observed. In the collection from Spangle, Washington, 140 out of 164 promycelia had the nuclei in two groups. In two other collections the nuclei were aggregated into two groups in less than half of the 52 promycelia observed. Although these observations suggest that dwarf-bunt collections may be specialized in this respect, there is need for further study of the nuclear behavior.

The reaction of the variety Orfed (C.I. 11913) to dwarf bunt at Troy, Idaho, and Bozeman, Montana, suggests specialization with respect to mor-

FIG. 3. Photograph showing difference in type of smutted head and smut balls produced by two collections of dwarf bunt on Orfed wheat. Center, healthy. Left, smutted head from Bozeman, Montana. Right, smutted head from Troy, Idaho.

phology of smutted heads and smut balls. Most of the smutted heads of this variety at the former location are slender and the smut balls are small, while at the latter location more vigorous smutted heads and smut balls are formed (Fig. 3). Thus, on the basis of this difference, together with the differences in chlamydospore morphology and rate of spore germination already mentioned, these two collections may be regarded as physiologic races.

It seems clear from the results presented here that there are specialized entities of *Tilletia caries* which cause dwarf bunt. These are not distinguished on the basis of pathogenicity and there is no evidence to indicate that pathogenic races do exist. However, since races do exist which are

distinguishable by other characters it seems probable that there also are pathogenic races.

Breeding for Dwarf-Bunt Resistance

Breeding for dwarf-bunt resistance in winter wheats has been a major phase of the wheat improvement program in the Pacific Northwest for many years. Relief (C.I. 10082), the first resistant variety to be developed, was distributed by the Utah Agricultural Experiment Station in 1934 (13). Four other dwarf-bunt-resistant varieties have been released since that time. These are listed in table 3. Three of the resistant varieties (Relief, Cache, and Wasatch) are hard red winter wheats. The other two, Rex and Hymar, are soft white wheats, representing the common and club types, respectively. These five varieties have a sufficiently broad geographic adaptation to cover the dwarf-bunt-infested Pacific Northwest.

TABLE 3.—*Dwarf-bunt-resistant varieties released for commercial production*

Variety	C.I. Number	Pedigree	Source of resistance	Releasing station
Relief	10082	Hussar × Turkey (Utah No. 26)[a]	Hussar	Utah
Cache	11599	Ridit × Turkey (Utah No. 26)	Ridit	Utah
Wasatch	11925	Relief × Ridit	Relief, Ridit	Utah
Rex	11689	White Odessa × Hard Federation	White Odessa	Oregon
Hymar	11605	Hybrid 128 × Martin	Martin	Washington

[a] Selection from Turkey, C.I. 2996, which has no known factors for resistance to bunt.

However, these are not perfect wheats and there is a continuous effort to develop better varieties and still maintain dwarf-bunt resistance.

Investigations and practical experience have demonstrated that dwarf bunt is most effectively controlled by the Martin-Hussar gene group (3, 8, 12). In addition, moderate resistance to dwarf bunt is found in Ridit but the protective degree of this resistance is not sufficient under severe epiphytotic conditions (9). For this reason the former type of resistance is more important than the latter, in breeding wheats with high resistance to dwarf bunt. The practical implications of this established fact are well illustrated in the results of wheat breeding in the Pacific Northwest in recent years (Table 4).

The first 24 hybrid selections listed in table 4 carry the Martin-Hussar type of resistance and are highly resistant to dwarf bunt. The next three carry the Ridit type of resistance and are moderately resistant to dwarf bunt. The last two selections listed in table 4, which carry only the Turkey (4) type of resistance, are highly susceptible to dwarf bunt. In the light of this information, therefore, the Martin gene appears to be the most important resistance factor in the development of dwarf-bunt-resistant varieties.

There is some evidence that Wasatch carries a combination of resistance factors that give a higher degree of resistance to dwarf bunt than does the Martin gene. Wasatch almost invariably has less dwarf bunt than do varieties with only the Martin type of resistance. In a test at Bozeman, Montana, in 1946 Wasatch had 0.5 per cent of dwarf bunt while Hussar (C.I. 4843), Martin (C.I. 4463), White Odessa (C.I. 4655), and Rex (C.I. 10065) had 2, 2.5, 3, 5, and 7 per cent, respectively. This apparent differ-

TABLE 4.—*The reaction to dwarf bunt of 29 hybrid selections of winter wheat, showing the relative value of three types of bunt resistance in breeding dwarf-bunt-resistant varieties*

Type of resistance, and hybrid	Selection number	C.I. number	State of origin	Reaction to dwarf bunt
Martin-Hussar Resistance				
Ridit × Relief	122a–327–1	11925	Utah	
Do	122a–70–3	11908	Do	
Do	112a–72–7	11909	Do	
Do	122a–20–3	11904	Do	
Do	122a–394–4	11905	Do	
Rex × Rio	381958	12234	Oregon	
Do	382165	12242	Do	
Do	381951	12246	Do	
Rex × Oro	382241	12421	Do	
Rio × Rex	382041	12422	Do	
Alicel × Rex	P-23	.....	Do	Highly resistant
Do	P-C16	.....	Do	
Do	P-C172	.....	Do	
Do	P-80	.....	Do	
Yogo × Wasatch	.....	.....	Montana	
Martin × Tenmarq	.....	11804	Texas	
Do	.....	11823	Do	
Do	.....	11824	Do	
Turkey (C.I. 11530) × Oro[a]	.....	.....	California	
Brevon × Relief	109–45	.....	Idaho	
Do	366–45	.....	Do	
Brevon × Wasatch	754–45	.....	Do	
Hussar × Kanred	105	11981	Nebraska	
Do	31B–96	11989	Do	
Ridit Resistance				
Brevon × Carlson's Fife	700–45	.....	Idaho	Moderately resistant
Ridit × Utah Kanred	.....	11599	Utah	
Composite Cross[b]	1–3–11–5	12385	Washington	
Turkey Resistance				
Alicel × Oro	P-10	12247	Oregon	Highly susceptible
Rio × Alicel	P-47	12377	Do	

[a] Thirteen of the selections from this cross that were tested were highly resistant to dwarf bunt.

[b] Brevon × (Oro × Turkey-Florence) (Oro × Fortyfold-Federation). Fifteen selections from this cross have been tested and found to be moderately resistant, thus reflecting at least the Brevon (Ridit) type of resistance.

ence, though only slight and of little practical significance, has been observed repeatedly under conditions of severe infestation. When infection percentages on susceptible varieties are relatively low (less than 50 per cent), this difference usually is not evident.

Plant breeders have been highly successful in the development of dwarf-bunt-resistant winter wheats. Some of the most promising selections are among those from Martin × Tenmarq, and Turkey (C.I. 11530) × Oro. If quality tests prove satisfactory, a new dwarf-bunt-resistant variety for Montana can be selected from this group. Most of these resistant lines, however, are not well suited to commercial production, and the problem remains of combining resistance to dwarf bunt with as many other desirable characteristics as possible.

Control Practices

Wherever dwarf bunt has occurred in destructive proportions one or more resistant varieties have been introduced to curb its onslaught. In Utah and certain parts of southern Idaho the introduction of Relief and Cache served to reduce the incidence of dwarf bunt from devastating proportions to almost negligible amounts. These varieties were largely replaced by the more resistant Wasatch. More recently, however, Cache has been increasing again because it yields slightly better and is more resistant to shattering than is Wasatch.

In the Gallatin and Flathead valleys of western Montana the dry-land winter-wheat industry was threatened with possible complete elimination by dwarf bunt in the period 1934–40. The introduction of Cache and Wasatch from Utah brought about a marked reduction in the amount of this disease within three years and, in fact, virtually saved the winter-wheat industry of that area.

The need for higher resistance than is carried by Ridit and Cache is well illustrated by the experience of introducing Cache into the Gallatin Valley of Montana. Under the severe conditions of infestation occurring in this area the highly susceptible variety Montana 36 had upwards of 75 per cent of dwarf bunt and Cache had only about 30 per cent in the most heavily infested fields. However, Wasatch under similar conditions had only a trace of dwarf bunt, thus demonstrating the superiority, under commercial conditions, of its resistance to this disease over that of Cache.

The most recent example of dwarf-bunt control through the introduction of a resistant variety is found in the infested area of Douglas County, Washington. Dwarf bunt is known to have been present in that area since 1940. It occurred in small amounts every year after that until 1945 when it reached severe proportions in at least two fields, one of Golden and the other of the Rio variety. Wasatch was introduced from Montana for seeding in 1946 and it proved to be satisfactory, both in field adaptation and in its resistance to dwarf bunt. Again the superiority of this type of resistance over the Ridit type was demonstrated. Adjacent plantings in the same field showed 10 per cent dwarf bunt in Ridit, as against traces in Wasatch. In 1948, when dwarf bunt reached destructive proportions in Rio and other susceptible varieties in this area, Wasatch was vir-

tually smut-free and in other respects appeared to be as good as or better than Rio, the predominant variety in that area.

SUMMARY

Dwarf bunt can seldom be reproduced artificially by seed inoculation. Repeated inoculations over a period of 15 years at experiment stations in the Pacific Northwest gave negative results in all cases except one.

Dwarf-bunt inoculum persists in the soil for two or more years, as shown by its occurrence in commercial wheat fields and by controlled experiments.

Dwarf bunt occurs in a wide range of climatic conditions and soil types. It is more prevalent and severe in dry-land areas but also attacks irrigated wheat. A compact soil favors the development of dwarf bunt.

The height of plants infected with dwarf bunt is only one-third to one-half that of noninfected plants, depending upon the variety. Among the varieties studied, the degree of dwarfing was greatest in Turkey and least in Triplet.

There is no evidence that pathogenically specialized races of dwarf bunt occur in the Pacific Northwest. Specialized entities based on chlamydospore germination, size of spores, prominence of spore wall reticulation, number of primary sporidia produced, branching of the promycelia, nuclear behavior, and host morphology were identified.

Only those varieties carrying the Martin-Hussar gene complex are highly resistant to dwarf bunt. Varieties with the Ridit type of bunt resistance are moderately resistant to dwarf bunt, while those with the Turkey factor (TT) are highly susceptible.

Plant breeders have been highly successful in the development of agronomically desirable wheats with resistance to dwarf bunt. Among commercial wheats in production in the Pacific Northwest, Relief, Wasatch, Cache, Hymar, and Rex are resistant to this disease. Many hybrid selections with dwarf bunt resistance are now available for breeding stock and some of these have value as potential new varieties.

Dwarf bunt can be controlled only by growing resistant varieties. This disease has been successfully controlled in Utah, southern Idaho, western Montana, and central Washington by the introduction of varieties with high resistance.

AGRICULTURAL EXPERIMENT STATIONS OF IDAHO, MONTANA,
UTAH, AND WASHINGTON

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EFFECT OF FERTILIZATION ON THE RECOVERY OF TRANSPLANTED SUGAR-BEET SEEDLINGS AFFECTED WITH APHANOMYCES COCHLIOIDES DRECHS. IN THE GREENHOUSE[1]

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INTRODUCTION

It is very important to have vigorous young beet seedlings prior to thinning in order to obtain a good stand. This is especially true when mechanical thinning of sugar beets is practiced. Very often a poor stand of sugar beets results because of seedling diseases or so-called "black root" of sugar beets.

Under Montana conditions when black root is severe, some beet seedlings die, although a large percentage of them recover. Damage to the crop, therefore, is primarily due to the retarded growth of plants of low vigor.

Extensive studies over a period of several years to determine the cause of seedling disease of sugar beets in Montana (1) showed that, although several different organisms may be involved in producing seedling disease of beets, *Aphanomyces cochlioides* is responsible for the major percentage of this disease in Montana.

In investigations relative to the occurrence and control of seedling diseases of sugar beets (2, 3, 4, 7) conducted in Montana, the effect of seed treatments, proper and balanced fertilization, preceding crops to sugar beets, temperature, and moisture were studied. It was found that a proper and balanced fertilization of the soil was the most important factor in the control of seedling diseases in Montana. Only small amounts of seedling diseases occurred in sugar beets planted in well fertilized soil, while large amounts occurred in soil low in the necessary nutrients.

Seed treatment was found to be only slightly beneficial in controlling seedling diseases of beets. The lowest amount of seedling disease occurred when beets were planted after corn; the highest, when beets followed beets. Diseases occurred in increasing amounts when beets were planted after corn, potatoes, oats, alfalfa, beans, and beets. Low temperatures and optimum moisture are more favorable to sugar beets from the standpoint of freedom from seedling diseases than are high temperatures and high moisture.

Since soil nutrients unquestionably have a great effect on the control of seedling diseases of sugar beets, it was decided to investigate the effects

[1] Contribution from Montana Agricultural Experiment Station, Paper No. 215, Journal Series.

of various nutrients and their combinations on the recovery of diseased beets and their subsequent yield under greenhouse conditions.

MATERIALS AND METHODS

Soil from the third-year alfalfa plot in Rotation 64 at the Huntley Branch Station, Huntley, Montana, was used in greenhouse flats holding about 24 lb. of soil. A high percentage of seedling diseases always occurred in this type of soil in previous studies. Four rows of segmented sugar-beet seeds were planted in several greenhouse flats. After approximately 1 month of growth the beet seedlings were carefully dug, washed, and graded into healthy, slightly diseased, and severely diseased plants. The graded beet seedlings were planted in nine soil flats filled with the same soil, to which the following fertilizers were applied:

1. N
2. P
3. K
4. M
5. NP
6. PM
7. NPK
8. NPKM
9. Check

TABLE 1.—*Effect of fertilization on recovery of transplanted diseased sugar-beet seedlings in the greenhouse, Bozeman, Montana, 1948*

Soil treatment	Degree of disease of transplanted seedlings	Data on harvested plants				
		Number healthy	Av. height	Weight of single plant		Color of plants
				Top	Root	
			cm.	*gm.*	*gm.*	
N	Healthy	6	9	6.3	0.5	dark green
	Slight	6	8	3.7	0.3	dark green
	Severe	6	9	6.2	0.5	dark green
P	Healthy	6	13	5.7	1.7	yellow green
	Slight	5	13	5.2	1.7	yellow green
	Severe	6	13	5.0	1.2	yellow green
K	Healthy	6	7	2.9	0.6	light green
	Slight	6	8	2.7	0.3	light green
	Severe	6	7	1.6	0.2	light green
M	Healthy	6	17	12.3	2.9	light green
	Slight	5	15	6.6	1.9	light green
	Severe	6	13	6.8	1.8	light green
NP	Healthy	6	25	41.7	5.2	green
	Slight	6	24	20.5	1.5	green
	Severe	6	25	28.0	2.2	green
PM	Healthy	6	16	9.7	3.3	light green
	Slight	5	14	7.4	2.6	light green
	Severe	6	14	8.0	2.3	light green
NPK	Healthy	6	34	43.0	3.5	green
	Slight	6	29	46.3	4.0	green
	Severe	4[a]	22	25.4	1.8	green
NPKM	Healthy	6	36	68.3	6.2	green
	Slight	6	23	20.5	1.0	green
	Severe	6	26	40.8	4.0	green
Check	Healthy	6	8	3.5	0.3	green
	Slight	6	6	2.2	0.3	green
	Severe	6	5	1.7	0.3	green

[a] One plant was diseased at harvest time.

Nitrogen (N) was used in the form of sodium nitrate in the amount of 31.4 gm. per flat; phosphorus (P) in the form of treble superphosphate in the amount of 3.6 gm. per flat; potassium (K) in the form of potassium chloride in the amount of 9.4 gm. per flat; and manure (M) in the amount of 1/5 of soil volume. Fertilizers were added in a dry form and thoroughly mixed with the soil.

Three rows of six sugar-beet seedlings each were planted in each flat of soil. One row was planted with healthy beet seedlings, the second with slightly diseased, and the last row with severely diseased seedlings. Water was added on the surface until the soil was uniformly moist.

Beets were grown in these flats approximately 2 months. At the end of this period the beets were dug and washed. Readings were taken on the number of healthy and diseased beet seedlings harvested. The height and weight of beets from each row was determined and their color was recorded. The results of these readings are presented in table 1.

RESULTS AND DISCUSSION

The results show that practically all diseased beets recovered. Similar results were obtained in Canada (6). Since the symptoms of diseased seedlings closely resembled those described for *Aphanomyces cochlioides* (1), it was assumed that this organism was mainly responsible for the disease. There was slight mortality in a few flats, and one beet remained diseased in a row planted with severely diseased seedlings in the soil fertilized with NPK.

The greatest difference in behavior of these beets was the amount of growth due to the different fertilizers added. The weight of the beets grown in soils fertilized with nitrogen, phosphorus, and potassium alone was slightly greater than the weight of those in the check. A more pronounced increase in weight occurred with the addition of manure and manure and phosphorus to the soil, and the greatest increase was with NP, NPK, and NPKM, especially the last (Table 1). Beets in the check remained very small. It has been shown that increasing the fertility of the soil, particularly with respect to phosphate, greatly reduces the losses due to *Aphanomyces cochlioides* Drechs. (5).

The tops of all transplanted seedlings grew more than the roots, which is natural since sugar beets develop leaves during the first part of their vegetative period and the root begins to increase in size later.

Healthy transplants, in practically all cases, produced the heaviest tops and roots. In some instances the rows planted with severely diseased seedlings produced plants heavier than those planted with slightly diseased seedlings. This was because of the crowding of the plants in the flats, with healthy and severely diseased seedlings in the outside rows and the slightly diseased seedlings in the middle rows.

The tops of the harvested beets varied from yellow-green to deep green in relation to the supply of nitrogen available to the plants.

Although the diseased sugar-beet seedlings showed a remarkable ability for recovery, this recovery was always delayed and hindered by the lack of available nutrients in the soil. It appears that if fertility of the soil is low and the beet seedlings have a considerable amount of disease, their condition may be improved by early application of nutrients.

This experiment was repeated twice with practically the same results. The result of only one experiment is reported.

SUMMARY

One-month-old diseased sugar-beet seedlings grown in the greenhouse and transplanted to soil flats fertilized with various combinations of nitrogen, phosphorus, potassium, and manure showed remarkable ability for recovery, especially in flats which received complete fertilization or manure. A row of healthy beet seedlings and two rows of diseased seedlings were planted in each flat of soil. Diseased seedlings grown in well-fertilized soil produced large plants. The seedlings grown in soil fertilized with nitrogen, phosphorus, and potassium alone, or grown in the check soil, remained very small.

These experiments show that if the fertility of the soil is low, and the beet seedlings have a considerable amount of disease, their condition may be improved by an early application of nutrients.

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FACTORS AFFECTING THE SURVIVAL OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN SOIL[1]

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It has long been known that soil cropped continuously to flax is likely to become "wilt-sick" because *Fusarium lini* Bolley accumulates and persists in the soil. On the other hand, *Helminthosporium sativum* P., K., and B. does not seem to accumulate to so great an extent. The contrast between the behavior of the two organisms in the soil is very conspicuous in two contiguous plots at University Farm, St. Paul, Minnesota. One has been cropped continuously to flax since 1912, and the other has been cropped continuously to barley since 1920. Abundant inoculum has been added to these two plots periodically. The flax plot has become so thoroughly infested that 100 per cent of the plants of susceptible varieties always become infected and usually almost 100 per cent are killed. On the contrary, barley still grows well in the Helminthosporium plot despite attempts to attain thorough infestation of soil with *H. sativum*.

There is limited information regarding the survival of *Helminthosporium sativum* and *Fusarium lini* in soil. Bolley (3) and Bolley and Manns (4) reported that *F. lini* is the principal cause of soil sickness, and that it accumulates in the soil with successive cropping of flax. Sanford (11) and Henry (7) have stated that very little disease occurred on wheat or barley in the fields artificially inoculated with *H. sativum*.

Henry (6) found that the natural microflora of the soil had a marked inhibitive effect on the growth of *Helminthosporium sativum*. Sanford and Cormack (12) demonstrated that the addition of certain fungi, bacteria, and Actinomycetes to the soil reduced the destructive effects of *H. sativum*. No extensive work has so far been reported, however, on the relation of soil microflora to the development of flax wilt and the accumulation of *F. lini* in soil.

The writer therefore attempted to find out why *Fusarium lini* develops and persists in the soil, while *Helminthosporium sativum* does not. As the soil type of the two University Farm plots is the same and the weather conditions obviously are the same, and as the cropping history is so well known, the two plots offered exceptional opportunity for comparative studies.

MATERIALS AND METHODS

Four races each of *Helminthosporium sativum* and *Fusarium lini* were

[1] A condensed version of a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, June, 1949.

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isolated from diseased barley and flax plants, respectively, and were designated as races on the basis of differences in pathogenicity and cultural characters. These races[2] are referred to as races 1, 2, 3, and 4. A mixture of the four races of each pathogen was used throughout the investigation, unless otherwise indicated.

Three varieties of barley (Barbless C.I. 5105 (Wisconsin 38), Mars C.I. 7015, and Peatland C.I. 5267), and three varieties of flax (Crystal C.I. 982, Redwing C.I. 320, and Punjab C.I. 1115) were used.

Selected seeds of barley were treated in 1:1000 aqueous solution of New Improved Ceresan for 30 min. and then washed in running water and dried. The flax seeds were treated with New Improved Ceresan dust at the rate of 1.5 oz. per bu. of seeds. Only those lots which were found to have 95 to 100 per cent disease-free seeds in plating tests were used in the experiments.

Most of the greenhouse tests were made in a steamed soil comprising two parts loam and one part sand. The soil type of University Farm plots (barley plot and flax-wilt nursery) is Waukegan silty loam, with a pH about 5.7.

The effect of *Helminthosporium sativum* on barley was expressed as a disease index, which was computed after grouping the plants into the following five disease-severity classes: 1. Plants with no disease symptoms; severity, 0 per cent. 2. Plants with light brown lesions on the coleoptile; severity, trace to 30 per cent. 3. Plants with dark brown lesions on the coleoptile; severity, 31 to 60 per cent. 4. Plants severely stunted, crown and base of the plants partially decayed, and the root system partially destroyed; severity, 61 to 90 per cent. 5. Seedlings killed before or after emergence, root system, crown, and coleoptile completely destroyed; severity, 91 to 100 per cent.

The disease index was obtained by multiplying the number of plants in classes 1, 2, 3, 4, and 5 by 0, 15, 45, 75, and 95, respectively, summating all the resulting figures, and dividing by the total number of plants in all classes. The prevalence of *Fusarium lini* was determined by counting the number of plants wilted at the end of each experiment.

STUDY OF THE MICROFLORAL POPULATION OF THE SOIL

Isolations of fungi, bacteria, and Actinomycetes were made from the flax-wilt nursery and from the barley plot every month from June 1947 to May 1948, for the following purposes: 1. To determine the prevalence of *Helminthosporium sativum* and *Fusarium lini* in the barley plot and wilt nursery, respectively. 2. To obtain isolates of the most prevalent groups of fungi, bacteria, and Actinomycetes for the study of their effects on the growth of *H. sativum* and *F. lini*. 3. To study the seasonal variation in the

[2] The designation of races as 1, 2, 3, and 4 is arbitrary, and these numbers are not the same as those used by other investigators in previous works.

microbial population of the soil in relation to the population of *H. sativum* and *F. lini.*

The plate-dilution method was used for the isolation of soil microorganisms. Dilutions of 1:1000 for the quantitative study of fungus flora and 1:100,000 for the determination of bacterial population of the soils were found to be the most suitable. Waksman's synthetic medium was used for fungi and albumin agar for bacteria.[3] Five random soil samples were taken from each plot, and 10 replicated plates were used for each sample and each dilution. Fungal and bacterial colonies in the respective plates were counted, and the number of fungi and bacteria per gm. of soil determined (Table 1). Isolates from all colonies of *Fusarium* that appeared

TABLE 1.—*The numbers of microorganisms per gram of soil in the flax-wilt plot and the barley plot at approximately monthly intervals from June 29, 1947, to May 30, 1948, inclusive*

Date of isolation	Microorganisms					
	In millions		In thousands			
	Bacteria and Actinomycetes		Fungi			
			Wilt plot		Barley plot	
	Wilt plot	Barley plot	Total	*F. lini*	Total	*H. sativum*
1947						
June 29	191	149	31	2.8	29	0.0
July 28	226	167	29	2.4	26	0.01
Aug. 14	158	157	27	2.9	31	0.0
Sept. 10	336	281	82	4.5	55	0.01
Oct. 13	300	230	88	5.1	56	0.01
Nov. 16	156	159	86	3.8	48	0.0
Dec. 14	32	17	20	1.6	14	0.0
1948						
Jan. 14	41	16	25	1.4	17	0.0
Feb. 15	19	21	19	1.4	21	0.0
Mar. 29	224	221	52	3.8	47	0.0
Apr. 25	190	181	42	2.3	36	0.01
May 30	132	182	43	2.5	22	0.0

in the dilution plates of the soil samples taken from the flax-wilt nursery were tested for their pathogenicity on Punjab flax, in order to distinguish *F. lini* from other soil Fusaria. The pathogenicity of *Helminthosporium sativum* obtained from the barley plot was tested on Barbless barley.

The population of *Fusarium lini* in the wilt nursery averaged about 3,000 spores and mycelial fragments per gm. of soil, whereas only 4 colonies of *Helminthosporium sativum* were obtained from the barley plot.

The data in table 1 show that the population of soil microorganisms

[3] Waksman's medium: agar, 20 gm.; dextrose, 10 gm.; peptone, 5 gm.; potassium dihydrogen phosphate, 1.0 gm.; magnesium sulphate, 0.5 gm.; water, 1000 ml. Adjust to pH 4.0.

Albumin agar: agar, 15 gm.; powdered egg albumin, 0.25 gm.; glucose, 1 gm.; magnesium sulphate, 0.20 gm.; potassium monohydrogen phosphate, 0.50 gm.; ferrous sulphate, trace; water, 1000 ml. Adjust to pH 7.0.

differed in different months of the year. The population of bacteria and fungi was highest in September–October and lowest in January–February. There seems to be a general rise in March and then a gradual decline from April to August, with an increase to the maximum in September and October. The population of *Helminthosporium sativum* was generally low and no important fluctuations were evident.

These data indicate that the conditions favoring growth and multiplication of other organisms also favored *Fusarium lini*. It is evident, however, that *Helminthosporium sativum* either does not grow and multiply appreciably in the soil, or else its presence is not detectable with the techniques used. The isolation of *H. sativum* was no more frequent when a number of differential media was used or soil was plated directly than when the soil dilution method was used.

THE EFFECT OF SOIL MICROORGANISMS ON THE GROWTH OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN CULTURE MEDIA

In these tests 86 isolates of fungi, bacteria, and Actinomycetes obtained from the barley plot and flax-wilt nursery at University Farm were tested to determine their effect on the growth of *Helminthosporium sativum* and *Fusarium lini* on 1.5 per cent potato-dextrose agar, which was the most suitable medium for the growth of most of the organisms tested. Three different methods of testing were used: 1. The test organism was streaked in the center of a Petri dish and *H. sativum* or *F. lini* placed on each side of it. 2. The center of the Petri plate was inoculated with the test organism, and *H. sativum* or *F. lini* placed around it. 3. Spore suspensions of *H. sativum* or *F. lini* were poured in Petri plates and the test organism was streaked through the center of the plate. All experiments were made in triplicate.

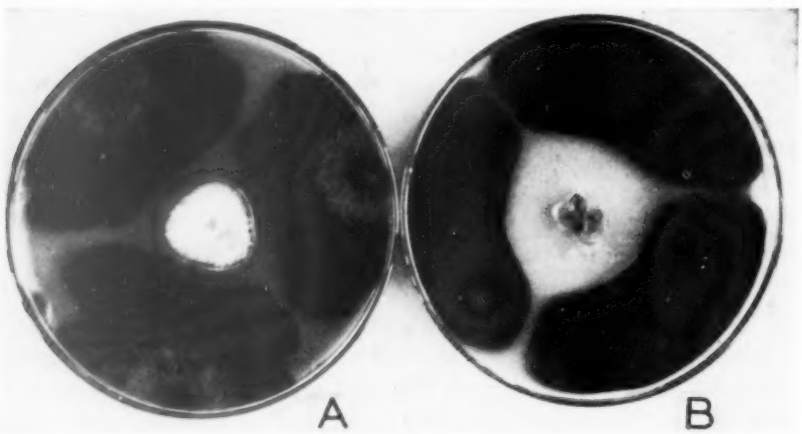

FIG. 1. A. Moderate antibiotic effect of *Penicillium* sp. on *Helminthosporium sativum* in culture. B. Strong antibiotic effect of *Bacillus subtilis* on *H. sativum* in culture.

The effects observed were classified according to the zones of inhibition evident in the plates. In class 0, no inhibition was observed. Classes 1, 2, 3, and 4 were based on inhibition zones of 1 mm., 1 to 3 mm., 3 to 5 mm., and more than 5 mm., respectively. Class 4 also was characterized by malformed hyphae of *H. sativum* and *F. lini* bordering the inhibition zone. Classes 0 and 1 represent slight effects, classes 2 and 3 moderate effects, and class 4 a strong effect (Fig. 1). The degrees of antibiosis are summarized in table 2.

TABLE 2.—*The degree of antibiosis of different isolates of species of bacteria, Actinomycetes, and fungi, against Helminthosporium sativum and Fusarium lini on potato-dextrose agar*

Antibiotic organism	No. of isolates tested	No. of isolates in each antibiotic class[a]									
		H. sativum					*F. lini*				
		0	1	2	3	4	0	1	2	3	4
Bacillus subtilis	34	10	0	4	3	17	21	3	6	4	0
Actinomyces spp.	12	6	0	4	3	0	11	1	0	0	0
Penicillium luteum	34	1	0	2	1	0	4	0	0	0	0
P. oxalicum	2	1	0	0	1	0	1	0	1	0	0
P. notatum	1	0	0	1	0	0	1	0	0	0	0
P. rugulosum	1	0	0	1	0	0	1	0	0	0	0
Penicillium sp.	2	1	0	1	0	0	1	1	0	0	0
Aspergillus fumigatus	6	2	0	3	1	0	5	0	1	0	0
A. flavus	2	1	0	1	0	0	2	0	0	0	0
A. niger	1	0	0	1	0	0	1	0	0	0	0
Aspergillus spp.	4	4	0	0	0	0	4	0	0	0	0
Trichoderma lignorum	4	0	0	2	2	0	3	1	0	0	0
Fusarium spp.	3	2	0	0	1	0	3	0	0	0	0
Other genera	10	10	0	0	0	0	10	0	0	0	0
Total	86	38	0	20	11	17	68	6	8	4	0

[a] 0 = no effect; 1 = inhibition in a zone about 1 mm. wide; 2 = zone of inhibition 1 to 3 mm. wide; 3 = zone of inhibition 3 to 5 mm. wide; 4 = zone of inhibition 5 mm. or more with malformation of the hyphae bordering the inhibition zone.

Of 86 isolates tested, 34 were bacteria, 12 were Actinomycetes, and 40 were fungus isolates. The last group consisted of 10 isolates of *Penicillium*, 13 of *Aspergillus*, and 4 of *Trichoderma*, while 14 isolates belonged to other genera. Of the 34 isolates of soil bacteria, 24 were antibiotic to *Helminthosporium sativum*, 17 being strongly antibiotic (class 4) and 7 moderately so (classes 2 and 3). Ten bacterial isolates were moderately antibiotic to *Fusarium lini* and 3 inhibited the growth slightly (class 1). The isolates of bacteria that were antibiotic to *H. sativum* and *F. lini* were Gram-positive spore formers of the *Bacillus subtilis* Cohn emend. Prazmowski group. Only 7 of 12 isolates of Actinomycetes were moderately antibiotic (classes 2 and 3) to *H. sativum* and only one slightly antibiotic to *F. lini*.

Of 23 isolates of *Penicillium* and *Aspergillus* tested, 13 were moderately antibiotic to *Helminthosporium sativum* and 3 to *Fusarium lini*. One isolate of *Penicillium* sp. slightly inhibited the growth of *F. lini*.

The 4 isolates of *Trichoderma lignorum* (Pers. ex Fries) Bisby were all moderately antibiotic to *Helminthosporium sativum*, whereas only one of them inhibited the growth of *Fusarium lini* in culture, and its effect was slight. One isolate of *Fusarium* inhibited (class 3) the growth of *H. sativum*. Among 8 other genera of fungi tested, none was antibiotic to either *H. sativum* or *F. lini*.

Further studies with the metabolic products of certain strains of *Bacillus subtilis* and species of *Penicillium*, *Aspergillus*, and *Trichoderma* have shown that the bacterial filtrates conspicuously inhibited the growth of *Helminthosporium sativum* when 1 ml. of the filtrate was mixed with 20 cc. potato-dextrose agar. Filtrates of two strains of *Trichoderma lignorum* and one of *Penicillium oxalicum* Thom. also inhibited the growth of *H. sativum;* however, there was no marked inhibition in the growth of *Fusarium lini* on agar plates to which staling products of the antibiotic organisms were added.

The results suggest that a relatively large number of soil microorganisms may be responsible for repressing the population of *Helminthosporium sativum* in the soil, whereas *Fusarium lini*, which seems to be inhibited by relatively few, may be better adapted to grow and persist in the soil.

THE EFFECT OF ANTIBIOTIC ORGANISMS ON THE PATHOGENICITY OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN STEAMED SOIL

There is considerable evidence that the pathogenicity of soil-borne species of *Helminthosporium* can be materially reduced by the antibiotic effect of other soil-borne organisms. Greaney and Machacek (5) were able to control root rot of wheat, caused by *Helminthosporium sativum*, by inoculating steamed soil with *H. sativum* and *Cephalothecium roseum* Corda. Bisby, James, and Timonin (2) found that root rot of wheat caused by *H. sativum* was significantly reduced by the addition of *Trichoderma lignorum* to the soil.

The writer experimented to determine the relative effects of *Bacillus subtilis*, *Trichoderma lignorum*, and *Penicillium* sp. on the pathogenicity of *Helminthosporium sativum* and *Fusarium lini* in the greenhouse. The antibiotic tests were made in steamed soil in two series of pots, one for *H. sativum* and the second for *F. lini*. Each series comprised five sets, each set having four 6-in. pots. Four of these 5 sets were inoculated with the respective pathogens. The fifth set, to which steamed oat-wheat mixture was added, served as control. Inoculum of antibiotic organisms was added to 3 of the 4 sets infested with the pathogens. The antibiotic organisms used were *B. subtilis*, *T. lignorum*, and *Penicillium* sp., respectively.

The inoculum of the organisms was increased on oat-wheat mixture; about one pint of inoculum of the antibiotic organism and one pint of the pathogenic organism were mixed and added to four 6-in. pots.

TABLE 3.—*The disease index on Barbless barley and percentage of wilt on Punjab flax grown in steamed soil inoculated with Helminthosporium sativum and Fusarium lini, respectively, and with 3 antibiotic organisms*

Treatment	Disease index on barley		Treatment	Percentage of flax wilt	
	Exp. 1	*Exp. 2*		*Exp. 1*	*Exp. 2*
Control	0.4	0.7	Control	0	0
H. sativum + *B. subtilis*	0.7	0.9	*F. lini* + *B. subtilis*	88	80
H. sativum + *T. lignorum*	2.9	3.4	*F. lini* + *T. lignorum*	85	68
H. sativum + *Penicillium*	2.9	2.8	*F. lini* + *Penicillium*	87	77
H. sativum	4.2	4.0	*F. lini*	86	74

Analysis of variance of "disease index" data

Variance due to	DF	Sum of squares	Mean square	F	Significance 5 per cent	Significance 1 per cent
Replications	1	0.0689	0.0689	0.14	7.71	21.20
Treatments	4	19.25	4.81	29.19**	6.39	15.98
Error	4	0.1707				

** Highly significant.

The 20 pots of each series, which comprised 4 replicates and 5 treatments, were randomized on a greenhouse bench at about 80° F., and a new random arrangement was made every 4 days.

FIG. 2. Barbless barley grown at 80° F. in steamed soil infested with (A) no microorganisms, (B) *Helminthosporium sativum* and *Bacillus subtilis*, (C) *H. sativum* and *Penicillium* sp., (D) *H. sativum* and *Trichoderma lignorum*, (E) *H. sativum*.

The results are summarized in table 3. *Bacillus subtilis* was effective in decreasing the attack of *Helminthosporium sativum* and provided practical control against the seedling blight. Although *Trichoderma lignorum* and *Penicillium* sp. reduced the root rot of barley, they did not prevent it entirely. (See figure 2.) The analysis of variance of barley data show highly significant differences between treatments.

Although there was no control of flax wilt in pots to which antibiotic organisms were added, the wilting of flax seedlings started a week later in pots to which *Trichoderma lignorum* was added than in pots to which *Fusarium lini* alone was added.

FIELD EXPERIMENTS

Experiments in the field tested the influence of *Bacillus subtilis*, *Trichoderma lignorum*, and *Penicillium* sp. on the pathogenic effects of *Helminthosporium sativum* and *Fusarium lini*. There are no definite reports of the control of Helminthosporium root rot of barley and wheat by adding antibiotic organisms to normal soil. Sanford and Cormack (12) found that soil bacteria, Actinomycetes, and fungi reduced the pathogenic effects of *H. sativum* in different degree. Their results were based on tests made in steamed soil. According to Waksman (13), Chudiakov reported the control of flax wilt in Russia by adding an antibiotic soil bacterium to flax-sick soil. Beresova and Naoumova (1) stated that partial control of flax wilt could be attained by treating the flax seeds with *Pseudomonas* and *Achromobacter*.

TABLE 4.—*Blight and wilt development and yields in Barbless and Mars barley and Crystal and Redwing flax grown in field plots infested with Helminthosporium sativum and Fusarium lini, and with Bacillus subtilis, Trichoderma lignorum, and Penicillium sp.*

Crop and treatment	Disease development[a]		Yield (bu./acre)[b]	
	Barbless	Mars	Barbless	Mars
Barley				
Control	L −	L −	21.4	22.4
H. sativum + *B. subtilis*	L +	L	20.7	20.2
H. sativum + *T. lignorum*	M −	M	16.1	17.9
H. sativum + *Penicillium* sp.	M	M	17.3	18.7
H. sativum	H +	H +	12.6	12.1
	Crystal	Redwing	Crystal	Redwing
Flax				
Control	11	5	12.0	12.7
F. lini + *B. subtilis*	38	26	9.2	12.9
F. lini + *T. lignorum*	43	31	8.8	11.0
F. lini + *Penicillium* sp.	45	22	8.6	12.4
F. lini	47	24	8.7	11.0

[a] Infection in barley was light (L), moderate (M), or heavy (H), with plus and minus variation within each class. Infection on flax was determined as percentage of plants wilted.

[b] Least significant difference: for barley yields = 4.31; for flax yields = 1.80.

Fungal and bacterial inocula were grown as described previously and one pint of inoculum of a pathogen was mixed with one pint of inoculum of a test organism for distribution in an 18-ft. row. Where a pathogen was used alone, one pint of inoculum was mixed with one pint of sterilized oat-wheat mixture; and in the checks, 2 pints of sterile medium were added to the soil. The 5 treatments were randomized within each block, 3 replicates being made. The varieties used were Barbless and Mars barley and Crystal and Redwing flax. The severity of root rot of barley was determined at the end of the experiment by examining the plants in each row and grading the infection in each row as light, medium, or heavy. The severity of flax wilt was determined by counting the wilted plants in each row of each treatment. Data on yield served as additional evidence of the effect of different treatments. The data were analyzed statistically. The results are presented in table 4. The rows of barley to which *Helminthosporium sativum* and an antibiotic organism were added had much lighter infection and yielded significantly more than those inoculated with *H. sativum* alone.

There was little evidence of antibiosis in the flax plot, as the severity of flax wilt in rows treated with antibiotic organisms and in those inoculated with *Fusarium lini* alone was not conspicuously different. However, *F. lini* alone caused twice as much wilt on Crystal as on Redwing and yield of flax seemed to be related to the percentage of plants wilted.

Tests were also made to find the effect of soil moisture on the antibiotic activity of microorganisms against *Helminthosporium sativum* and *Fusa-*

TABLE 5.—*Blight and wilt development in Barbless and Mars barley and Punjab and Redwing flax, respectively; in moist and dry soil infested with Helminthosporium sativum and Fusarium lini, and with Bacillus subtilis, Trichoderma lignorum, and Penicillium sp.*

Crop and treatment	Disease development[a]			
	Moist soil		Dry soil	
	Barbless	Mars	Barbless	Mars
Barley				
Control	L –	L –	L –	L –
H. sativum + *B. subtilis*	M	M –	L	L +
H. sativum + *T. lignorum*	M	M –	M –	M –
H. sativum + *Penicillium* sp.	M +	M –	M –	M –
H. sativum	M –	M –	H –	H
	Punjab	Redwing	Punjab	Redwing
Flax				
Control	16	3	14	7
F. lini + *B. subtilis*	100	16	82	28
F. lini + *T. lignorum*	100	11	70	18
F. lini + *Penicillium* sp.	100	8	77	21
F. lini	100	10	88	25

[a] Infection in barley was light (L), moderate (M), or heavy (H), with plus and minus variations within each class. Infection on flax was determined as percentage of plants wilted.

rium lini in the field. The soil treatments and the strains of antibiotic organisms used were the same as in the previous experiment. The antibiotic organisms did not control the wilt of flax in dry or in moist soil (Table 5). The antibiotic effect of *Bacillus subtilis* against *Helminthosporium sativum* was somewhat better in comparatively dry soil than in moist soil.

EFFECT OF TEMPERATURE ON THE SURVIVAL OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN SOIL

There are several reports in literature on the effect of soil temperature on the pathogenicity of *Helminthosporium sativum* and *Fusarium lini*. Jones, Johnson, and Dickson (8) reported that the maximum infection of wheat seedlings by *H. sativum* occurred at 30° C., and the highest percentage of flax wilted at soil temperature of 24° to 28° C. Jones and Tisdale (9) give 24° to 28° C. as the optimum temperature for flax wilt and 38° C. as the maximum. Apparently the effect of soil temperature on the survival of these organisms has not been investigated.

In these tests attempt was made to determine the prevalence and survival of *Helminthosporium sativum* and *Fusarium lini* in steamed and nonsterile soils. Steamed and nonsteamed soil from the barley plot and the wilt nursery were infested with race 4 of *H. sativum* and race 4 of *F. lini*, respectively. Each soil lot and a water suspension of mycelial fragments and spores of the appropriate actively growing pathogen were mixed thoroughly before distribution to individual tins and glass jars. These containers were then stored in incubators at 8° to 10° C., 20° to 22° C., 35° to 37° C., and 45° to 50° C. One lot was kept at −15° to 0° C., the temperature range out of doors at University Farm, from December 29, 1948, to January 12, 1949. Control lots of soil received no inoculum.

Sterile water had been added to adjust the moisture content to 8 per cent of the dry weight of the soil. Weighing the containers twice daily and adding sterile tap water at incubator temperature to make up for the weight lost by evaporation maintained the moisture at the 8 per cent level. The storage time was recorded as soon as the soil temperature reached the temperature of the storage incubators.

Three replicate containers of infested soil and one of noninfested soil were removed from each incubator every 3 days for 15 days. They were placed in the greenhouse at about 80° F., and 10 seeds of Punjab flax or Barbless barley were planted in each. Three replicate cans of freshly steamed soil and 3 of natural soil were newly infested with the respective pathogens at each 3-day interval and were planted with flax or barley. These cans served as additional controls and provided data on the disease reactions of plants grown in newly infested soil which could be compared with data for stored soils. The prevalence and survival of *Helminthosporium*

sativum and *Fusarium lini* in these soils was judged by the disease index for the root rot of barley and by the percentage of flax wilt (Table 6).

Between −15° C. and +22° C. both *Helminthosporium sativum* and *Fusarium lini* survived storage better in steamed soil, where there were fewer biologic competitors than in the natural field soil.

Helminthosporium sativum survived storage at 35° to 37° C. but was almost eliminated within 6 days when storage temperature was as high as 45° to 50° C. *Fusarium lini*, on the other hand, was rapidly eliminated from infested steamed soil stored at 35° to 37° C., but survived fairly well in the infested natural soil stored at that temperature. Higher temperatures were detrimental to the survival of *F. lini*.

TABLE 6.—*The amount of disease that developed on Barbless barley and Punjab flax in steamed and natural soils previously inoculated with 4 races each of Helminthosporium sativum and Fusarium lini, and then kept 3 to 15 days at temperatures ranging from −15° to 50° C. prior to planting*

Storage		Disease index for barley		Percentage of flax wilt	
Temperature in degrees C.	Time (days)	Steamed soil	Natural soil	Steamed soil	Natural soil
−15 to 0	3	3.3	0.9	96	45
	6	2.1	2.3	50	37
	9	2.8	1.7	76	55
	12	2.2	1.4	73	23
	15	2.2	1.5	81	33
8 to 10	3	3.2	1.3	96	71
	6	3.6	1.3	100	50
	9	2.0	1.9	95	69
	12	2.4	1.1	95	69
	15	1.5	1.6	100	55
20 to 22	3	2.7	1.1	93	83
	6	3.7	1.9	96	60
	9	2.0	1.4	100	89
	12	2.3	1.0	90	79
	15	1.8	1.4	100	95
35 to 37	3	3.1	2.1	36	43
	6	3.7	0.9	8	22
	9	2.5	1.9	0	50
	12	1.6	0.9	0	13
	15	1.8	1.0	0	25
45 to 50	3	1.9	1.2	11	0
	6	0.9	0.9	0	5
	9	0.0	0.0	0	0
	12	0.0	0.0	0	0
	15	0.0	0.0	0	0
Controls: Newly infested soil, not stored	3	3.9	1.6	96	91
	6	2.9	2.1	100	75
	9	3.9	1.9	95	84
	12	3.9	2.1	95	87
	15	2.8	1.8	100	100

Differences in the survival of *Helminthosporium sativum* and *Fusarium lini* are not very striking. Nevertheless, at higher temperatures *H. sativum* seems to have an advantage over *F. lini*. It is unlikely, however, that either summer or winter soil temperatures in Minnesota would be limiting factors in the survival of the two pathogens.

RELATION OF STUBBLE TO THE SURVIVAL OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN SOIL

Infested plant stubble was gathered from the barley plot and the wilt nursery and added to steamed soil in 1 : 10, 2 : 10, and 4 : 10 ratios. In another series, soil was taken from the barley plot and the wilt nursery, cleaned of all stubble, and added to steamed soil in the same ratios. Three sets of pots were used as controls. The first set consisted of steamed soil, the second set was steamed soil plus inoculum of *Helminthosporium sativum* or *Fusarium lini*, and the third set was steamed soil plus steamed flax or barley stubble.

Four replicates were used for each treatment. Twenty-five disease-free seeds of Barbless barley or Punjab flax were planted in each plot. Two weeks after the emergence of plants the percentage of flax wilt was recorded. The barley plants were dug after two weeks, washed, and classified for the determination of disease index.

Root rot of barley was heavier in pots to which stubble had been added than in those to which infested soil was added. The disease indices of barley plants in pots with 1 : 10, 2 : 10, and 4 : 10 ratios of stubble to soil were 2.2, 1.5, and 3.0, respectively. In a similar soil series without stubble but with similar ratios of infested soil to steamed soil, the disease indices of plants were 0.7, 0.7, and 0.9, respectively. This definitely indicates that *Helminthosporium sativum* survives better on stubble than in soil.

These results substantiate those of Padwick (10), who showed that Helminthosporium root rot of wheat was considerably increased when infested root systems of grasses were added to soil.

The differences in the percentage of flax wilt were also conspicuous, although in exactly the opposite direction. The percentages of flax wilt in stubble series for the three ratios of 1 : 10, 2 : 10, and 4 : 10 were 12, 19, and 25, respectively; whereas in the soil series the percentages were 44, 59, and 69, respectively. There was 30 to 40 per cent more wilt in steamed soil to which infested soil was added than in soil to which infested stubble was added. This indicates that *Fusarium lini* may survive better in soil than on plant refuse.

DISCUSSION

Fusarium lini accumulates in soils continuously cropped to flax, whereas *Helminthosporium sativum* does not seem to accumulate to the same extent in soils cropped to barley and wheat.

In the present studies it was found that the difference in the sensitivity

of *Helminthosporium sativum* and *Fusarium lini* to antibiotic organisms of the soil is one of the important factors accounting for the fact that *H. sativum* is not able to accumulate and infest the soil to the same extent as *F. lini*.

In spite of the fact that large amounts of inoculum of *Helminthosporium sativum* have been added to the barley plot every year for the last 18 years, only 4 colonies of *Helminthosporium sativum* were obtained from the barley plot in plating-out studies with soil dilutions; whereas there were 3000 colonies of *Fusarium lini* per gm. of soil from the flax-wilt plot to which inoculum of *F. lini* was occasionally added. On the other hand, the total number of colonies of all organisms per plate was approximately the same for the two plots. Having established the fact that *F. lini* is very abundant in the soil of the flax plot while *H. sativum* is very rare in the barley plot, the question naturally arose as to the reasons for these differences. It was found that *F. lini* is relatively unaffected by other microorganisms in the soil while *H. sativum* is deleteriously affected by many.

Of 86 isolates of bacteria, fungi, and Actinomycetes, 48 were antibiotic to *Helminthosporium sativum*, whereas only 12 were decidedly antibiotic to *Fusarium lini* and none of them was strongly antibiotic. Of the organisms that were antibiotic to *H. sativum*, several isolates of *Bacillus subtilis*, *Penicillium* spp., *Aspergillus* spp., and *Trichoderma lignorum* were most effective, as measured both by the width of the zone of inhibition and the final effect on the colonies.

Not only was the antibiosis of certain organisms effective on agar media but it was evident that some of the organisms could protect plants against infection in the field and greenhouse. When steamed soil was infested with *Helminthosporium sativum* and planted with barley, the plants were severely and uniformly diseased and stunted and yield was reduced significantly. When the soil was infested with a mixture of *H. sativum* and certain isolates of *Bacillus subtilis*, on the other hand, the barley plants grew almost as well as plants in noninfested soil and yield was not lowered significantly. *B. subtilis* therefore gave almost complete protection against the attack of *H. sativum*. Partial protection resulted also from the use of *Penicillium* species and *Trichoderma lignorum*, but the results were not so striking as those with *B. subtilis*.

Similar greenhouse and field experiments with flax wilt gave no significant differences in the amount of wilt regardless of the incorporation in the soil of organisms that had protected barley from blight as well as those that had been antibiotic to *Fusarium lini* in culture.

Soil moisture may affect the antibiotic activity of soil microorganisms. The antibiotic effect of *Bacillus subtilis* is operative against *Helminthosporium sativum* in relatively dry soil.

Both *Helminthosporium sativum* and *Fusarium lini* survive in the soil over a rather wide temperature range. Both organisms are almost elimi-

nated if soil temperature rises to 45° and 50° C. for 3 days, but they do seem to survive temperatures as low as – 15° C. for 15 days. Although the differences in the survival ability of the two organisms at these soil temperatures is very slight, *H. sativum* tolerates high temperature somewhat better than does *F. lini*. It seems improbable that temperatures as high as those used in these experiments would prevail often enough or long enough under natural conditions to account for the disappearance of either organism from the soil. What little advantage there is, however, seems to be with *H. sativum*. Consequently, these facts increase the probability that the effect of antibiotic organisms on *H. sativum* and their relative ineffectiveness on *F. lini* account to a considerable extent for the difference in survival ability in soil.

The limited survival of *Helminthosporium sativum* in the soil seems to be due to its ability to persist for some time on stubble, and it probably does survive on stubble that has been plowed under until antibiotic organisms reduce its ability to grow and multiply profusely in the soil.

Although factors other than antibiosis may influence the survival of *Fusarium lini* and *Helminthosporium sativum* in the soil, the conclusions seem justified that under the experimental conditions and in the soil type used by the writer, the difference in the sensitivity of the two organisms to antibiotic microflora in the soil is a major factor in the survival of the two organisms.

SUMMARY

The prevalence of *Fusarium lini* in the flax-wilt nursery to which inoculum of *F. lini* was occasionally added was 3000 spores or mycelial fragments per gm. of soil. On the other hand, the prevalence of *Helminthosporium sativum* in the barley plot, to which inoculum of *H. sativum* was added annually, was not more than 10 per gm. of soil and frequently this fungus could not be recovered from the plot. There seems to be a close association between the high counts of *F. lini* in the wilt nursery and the high incidence of flax wilt, and between the infrequent isolation of *H. sativum* and the comparatively low incidence of root rot.

The population of soil bacteria, soil fungi, and *Fusarium lini* varied with seasonal changes, being highest in September–October and lowest in January–February. The population of *F. lini* followed the same trend as that of other fungi and bacteria. This fact indicates that the conditions optimum for the growth of other soil microorganisms are optimum for *F. lini*.

Of a total of 86 isolates of bacteria, Actinomycetes, and fungi, 48 were antibiotic to *Helminthosporium sativum* and 12 to *Fusarium lini* when grown on potato-dextrose agar. The metabolic products of some of the bacteria, Actinomycetes, and fungi inhibited the growth of *H. sativum* when added to agar medium. The growth of *F. lini* was not conspicuously affected by the same products.

A soil isolate of *Bacillus subtilis*, when added to steamed soil with

Helminthosporium sativum, completely protected barley seedlings against root-rot. The *B. subtilis* also protected field plots of barley from heavy infection by the *Helminthosporium*. *Trichoderma lignorum* and *Penicillium* spp. also gave partial protection from the disease. Similar treatments failed to protect flax from wilt.

Barley grown in rod rows infested with mixtures of *Helminthosporium sativum* and one of the three antiobiotic microorganisms outyielded barley in the rows infested with *H. sativum* alone.

The antibiotic effect of *Bacillus subtilis* was operative against *Helminthosporium sativum* in relatively dry soil. The flax wilt was not controlled by the antibiotic organisms in either dry or moist soils.

Helminthosporium sativum survived temperatures of 45° to 50° C. for 6 days, both in steamed and natural soils. *Fusarium lini* was less able to survive high temperatures.

Addition of infested barley stubble to the soil increased the incidence of root rot more than did addition of soil infested with *Helminthosporium*. Addition of infested flax stubble to the soil increased the incidence of flax wilt but was less effective than the addition of soil infested with *Fusarium lini*.

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THE PATHOGENICITY OF SPECIES OF HELMINTHOSPORIUM ON CORN[1]

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Diseases of corn caused by *Helminthosporium turcicum* Pass. (4, 7) and *H. maydis* N. and M. (5) have long been known. Recently another species, *H. carbonum* Ullstrup, has become prevalent and destructive in the corn belt of the United States (11). Although species of *Helminthosporium* have been reported on corn in Minnesota, their relative prevalence and importance on different lines and varieties of corn and other gramineous hosts have not heretofore been studied. This paper presents preliminary results of work on this problem.

SOURCE AND IDENTITY OF HELMINTHOSPORIA

Helminthosporium carbonum, *H. sativum*, *H. turcicum*, and a long-spored isolate of *Helminthosporium*, designated in this paper as *Helminthosporium* Z[2], were isolated repeatedly from lesions on the leaves of corn obtained from several localities in Minnesota. Of the species isolated, *H. sativum* P., K., and B. was by far the most prevalent. Certain other fungi were frequently isolated, but were discarded as they were not pathogenic on corn. For a comparative study, several races of *H. sativum* from other sources were included in these tests.

Since *Helminthosporium* Z resembled *H. carbonum*, a detailed morphological study was made of monosporous isolates of the two. The spores used for comparison were produced on autoclaved wheat heads kept at room temperature (20° to 24° C.). Two hundred conidia of each isolate were measured 15 days after inoculation.

The conidia of *Helminthosporium* Z averaged 115 μ in length and 19 μ in width and the average number of septa was 9. They were from 36 to 238 μ long, although only a few spores longer than 200 μ were found. The width ranged from 10 to 26 μ, and the number of septa from 1 to 14. Typical conidia of *Helminthosporium* Z were usually slightly curved, elongate, and tapered slightly toward the rounded end. Conidia were dark olivaceous brown, and one or both ends might be subhyaline. Spores germinated in distilled water by the production of two polar germ tubes.

Conidia of *Helminthosporium carbonum* race 1 averaged 87 μ long and 21 μ wide, and had 8 septa. The difference in mean length of conidia of the

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[2] It seems desirable to designate the organism as *Helminthosporium* Z until there has been ample opportunity to study the range of variation within the species *H. carbonum*, as the organism may be an undescribed species of *Helminthosporium* or simply a variety or race of *H. carbonum*.

two Helminthosporium isolates was 25.80 $\pm$ μ; in mean width, 2.52 $\pm$ μ; and in the mean of the number of conidial septations, 0.99.

The symptoms produced by *Helminthosporium* Z resemble most closely those produced by races 1 and 5 of *H. sativum*[3]. Numerous small lesions, usually about 2 mm. in diameter and noncoalescent, are produced. The lesions are elliptical or oval, whitish to brownish, with a light-brown to brown border. The symptoms produced by *Helminthosporium* Z that are distinct from those produced by the other species of *Helminthosporium* are the chlorotic areas around the spots, and leaf curling and subsequent drying. Infected areas on the stems at first appear watersoaked but later become brownish. The organism also can cause rotting of the mesocotyl and roots. In this respect it behaves like *H. carbonum*.

The symptoms caused by *Helminthosporium sativum* on various cereals and grasses other than corn have been described by several workers (3, 4, 8, 10). In general, the most typical symptoms produced by *H. sativum* on corn resemble those mentioned for *Helminthosporium* Z. Races 4 and 5, however, differ from the other races of *H. sativum* and from *Helminthosporium* Z in the size of lesions produced on the leaf sheath. Fully developed lesions produced by races 4 and 5 of *H. sativum* are irregular to oval and colorless, transparent straw color, or chocolate brown with a brownish to purplish margin. The largest lesions were 12 mm. long and 3 mm. wide and they seldom coalesced. Symptoms produced on seedling leaves as well as the symptoms of root rot agree with those reported for *H. sativum* on other cereals and grasses (3, 10).

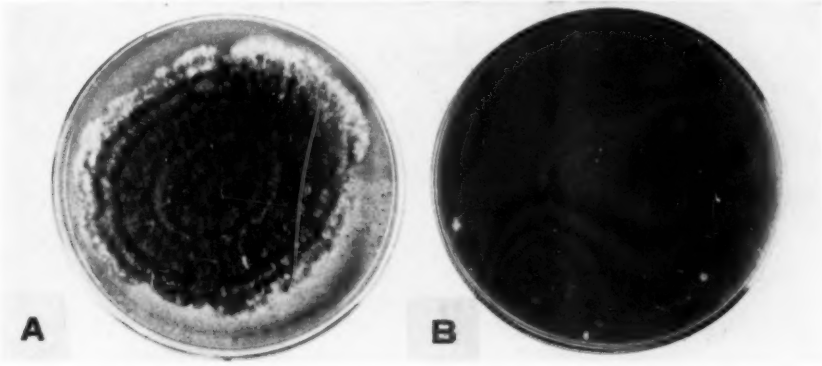

FIG. 1. *Helminthosporium* Z on two media: (A) potato-dextrose-corn steep agar, and (B) potato-dextrose-cornmeal agar.

Helminthosporium Z is extremely variable in cultural characters when grown on different nutrient media or under different environmental conditions (Fig. 1). Thus, *Helminthosporium* Z on one medium may resemble a race of *H. carbonum*, but may differ greatly on another.

[3] The numbers used in designating races of *Helminthosporium spp.* are not necessarily the same as those used by previous investigators.

PATHOGENICITY STUDIES

Relative Virulence of Species of Helminthosporium on Varieties of Corn in the Greenhouse

Preliminary pathogenicity tests were made with 26 cultural races of *Helminthosporium* on corn. From these tests, thirteen races were selected for more detailed study. Seed of five varieties of corn previously treated with Spergon were planted in autoclaved soil in 6-in. pots in the greenhouse. The inoculum was increased by growing the races for 20 days on 2 per cent potato-dextrose agar. Aqueous suspensions of inoculum were made by washing the cultures with sterile distilled water. This suspension was then strained through sterile cheesecloth and used to inoculate 30-day-old plants. Two methods of inoculation were tried: (a) injecting the inoculum into the second lowest internode with a hypodermic syringe, and (b) spraying the inoculum on the leaves with a small hand sprayer. After inoculation, the plants were kept for about 24 hr. in a muslin-covered moist chamber at approximately 27° C.

TABLE 1.—*Reactions of four varieties of corn to 13 races of Helminthosporium spp. when inoculated by injection and by spraying with conidial and mycelial suspensions in the greenhouse*

Race	Method of inoculation	Sweet corn[a]	Minhybrid 800	Minhybrid 500	Double cross D4
H. carbonum race 1	Injection	S	S	S	S
	Spray	S	S	S	S
Do 1–1[b]	Injection	MR	MR	MR	R
	Spray	MR	R	R	R
H. sativum race 1	Injection	MS	MS	MS	MR
	Spray	MR	MR	MR	MR
Do 2	Injection	MR	MR	MR	MR
	Spray	R	MR	MR	R
Do 3	Injection	MR	MR	MR	R
	Spray	R	R	R	R
Do 4	Injection	MS	MR	MR	R
	Spray	MR	MR	R	R
Do 5	Injection	MR	R	MR	R
	Spray	MR	MR	MR	MR
Do 6	Injection	R	MR	MR	R
	Spray	R	T	T	T
Do 7	Injection	MS	MS	MS	MS
	Spray	MR	MR	MR	R
Do 8	Injection	MR	R	R	R
	Spray	R	R	T	T
H. turcicum race 1	Injection	S	S	S	S
	Spray	S	S	S	S
Do 2	Injection	S	S	S	S
	Spray	S	S	S	S
Helminthosporium Z	Injection	S	MS	MS	MR
	Spray	MS	MS	MS	R

[a] S = susceptible; R = resistant; MR = moderately resistant; MS = moderately susceptible; T = trace.

[b] A variant of *H. carbonum* 1.

The plants were usually more severely infected when inoculated with a hypodermic syringe than when sprayed (Table 1 and Fig. 2.). In other experiments it appeared that when the inoculum was confined to the upper whorl of the plant, the infections were more or less alike irrespective of the method used.

Races 1 and 2 of *Helminthosporium turcicum* and race 1 of *H. carbonum* attacked all the varieties of corn about equally well. *Helminthosporium* Z infected all the varieties tested but the severity of attack was low on sweet

FIG. 2. Seedlings of sweet corn inoculated by means of hypodermic syringe with (A) *Helminthosporium sativum* race 7, (B) *Helminthosporium* Z, (C) *H. sativum* race 2, and check.

corn and on double cross D4 when the inoculum was sprayed on the leaves. There also were marked differences in pathogenicity among the races of *H. sativum:* Races 1, 2, 3, 4, 5, and 7 were moderately pathogenic on at least three varieties of corn, whereas races 6 and 8 were weakly pathogenic. A cultural variant of *H. carbonum* designated as *H. carbonum* race 1–1 was much less pathogenic than its parent, *H. carbonum* race 1. Therefore it also is a distinct parasitic race.

Reactions of South American Corn Selections to Two Races of Helminthosporium turcicum

Although races 1 and 2 of *Helminthosporium turcicum* were alike in pathogenicity in previous experiments, it was thought that some difference in virulence might be apparent on certain other varieties. Moreover, the reactions of South American corn selections to *H. turcicum* may be of practical importance. Ten South American selections were inoculated hypodermically with both races of *H. turcicum.* Sweet corn was included as a

susceptible variety and noninoculated plants of each selection were used as controls.

Selections 10, 15, and 17 of South American corn were resistant, moderately resistant, and moderately susceptible, respectively, to *H. turcicum*

TABLE 2.—*Reactions of ten selections of South American corn to two races of Helminthosporium turcicum*

Selection No. of corn	Source[a] of selection	Races and host reaction		
		Race 1	Race 2	Control
3	Brazil	S	S	O
4	Do	S	S	O
7	Do	S	S	O
15	Do	S	MR	O
17	Do	S	MS	O
10	Brazil and Peru	S	R	O
12	Do	S	S	O
14	Do	S	S	O
8	Chile	S	S	O
16	Do	S	S	O
Sweet corn	Minnesota	S	S	O

[a] The seed corn from South America was furnished by Mr. George Hafstad of the Rubber Development Corporation, Amazon Division, Brazil.

race 2, while race 1 attacked all selections equally well (Table 2). It is noteworthy that race 1 attacked all the varieties of corn tested whereas race 2 did not. Evidently, therefore, races 1 and 2 of *H. turcicum* can be considered as distinct parasitic races.

Inoculations on Miscellaneous Gramineae

The host range of those species of *Helminthosporium* that attack corn is imperfectly known, but this information may be important in control practices. To obtain more definite information on this subject, the writer inoculated a number of crop plants with four species of *Helminthosporium* known to be parasitic on corn.

Helminthosporium turcicum has been reported on various wild and cultivated species of the Gramineae (1, 2, 4, 6, 7, 9) as well as on corn. At present *H. carbonum* has been reported only from corn and nothing is known about the host range of *Helminthosporium* Z. *H. sativum* is known to attack the common small grains and many wild grasses (3, 10). Extensive cross inoculations were made with 13 races of *Helminthosporium* spp. Clean and surface-disinfected seeds of 6 species of grasses were planted in 4-in. pots filled with autoclaved soil and placed in the greenhouse at about 26° C. The seeds were submerged for 10 min. in a 1 : 1000 solution of bichloride of mercury mixed with a wetting agent (Nacconol), then rinsed in a 0.5–1.0 per cent solution of chlorinated lime.

The 15-day-old seedlings of the various cereals and grasses were sprayed with conidial suspensions of the various races and incubated in a moist chamber for about 24 hr. The substrate used for increasing the inoculum

was a half-and-half mixture of autoclaved wheat and oat seeds. The results, summarized in table 3, show that the host range varies considerably with the species and race of *Helminthosporium*. *H. turcicum* and *H. carbonum* infected three of the Gramineae. Four of the races of *H. sativum* infected

TABLE 3.—*Reactions of 6 species of Gramineae to 13 races of Helminthosporium spp.*

Species and race	Host and reaction[a]					
	Marquis wheat	Bonda oats	Barbless barley	Golden Bantam corn	Teosinte	Sudan grass
H. carbonum race 1	O	O	O	S	S	–
Do 1–1	–	–	–	MR	O	T
H. sativum race 1	MR	O	R	MR	O	MS
Do 2	O	O	R	R	O	MR
Do 3	MS	O	MS	R	R	S
Do 4	MS	O	S	MR	R	S
Do 5	S	T	T	MR	R	S
Do 6	MR	O	R	R	O	MS
Do 7	T	O	MS	MR	MR	MR
Do 8	O	O	O	R	O	T
H. turcicum race 1	O	O	R	S	S	S
Do 2	O	O	O	S	MS	S
Helminthosporium Z	O	O	O	MS	R	S

[a] S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant; T = trace; O = immune; – = no test.

at least five of the six hosts, whereas race 2 attacked barley and corn weakly and was moderately pathogenic on Sudan grass.

Helminthosporium Z was only weakly pathogenic on teosinte, while race 1 of *H. carbonum* infected it severely. However, the variant 1–1 of this species did not attack teosinte. All the species of *Helminthosporium* attacked Sudan grass; none of them attacked oats except race 5 of *H. sativum*, which attacked oats weakly.

Field Experiments

The pathogenicity of several species of *Helminthosporium* was determined on 8 varieties of corn and one of teosinte in the field. Duplicate tests were made in July, 1946. Each plot consisted of 9 rows, 130 ft. long and 3 ft. apart. Eight varieties of corn and one variety of teosinte were planted. Two seeds were sown per foot and each plot was divided into 13 blocks, 12 of which were inoculated with a race of *Helminthosporium* by injecting a conidial suspension into the spiral of the corn plant shortly before tasseling. Check plants in the 13th block were injected with sterile broth. The organisms were increased on an autoclaved half-and-half mixture of wheat and oats. At the time of inoculation the plants were from 37 to 41 days old and from 2 to 4 ft. in height, depending on the variety and location of plot. Results are given in table 4.[4]

[4] The seed corn was furnished by the Division of Agronomy and Plant Genetics, University of Minnesota, and seed of teosinte by Dr. J. G. Harrar of the Rockefeller Foundation, Mexico.

Although the weather was cold and dry after inoculation, the results agreed well with those in the greenhouse except that *Helminthosporium turcicum* race 1 appeared much less virulent in the field than in the greenhouse. This apparent loss in pathogenicity may be due to mutation, as there had been obvious changes in cultural characters on artificial media.

There were not only clear-cut differences in the virulence of the various

TABLE 4.—*Field reactions of eight varieties of corn and one of teosinte to 12 races of Helminthosporium spp. when inoculated by the hypodermic method in the field*

Species and race	Host[a] and reaction[b]								
	Double crosses							Golden Bantam	Teosinte
	408	500	505	602	800	W240	D4		
H. carbonum race 1	S	S	S	S	S	MS	S	S	S
H. sativum race 1	MS	MS	MS	MS	MS	MR	R	MS	R
Do 2	MR	MS	MR	R	MR	MR	MR	MR	R
Do 3	R	MR	MR	MS	MR	R	MR	MR	MR
Do 4	S	MS	S	S	MS	MR	MS	MS	MS
Do 5	S	MS	MR	MS	MR	R	MR	MR	R
Do 6	MS	MS	MS	MS	MR	MR	MR	R	MR
Do 7	S	S	S	MR	MS	MR	MS	MS	MS
Do 8	MS	MR	MS	MR	MR	R	R	MR	R
H. turcicum race 1	MS	S	S	MS	S	S	S	S	S
Do 2	MS	MS	MS	MS	MR	R	S	MR	MS
Helminthosporium Z	S	MR	S	MS	MS	MS	S	S	R

[a] Varieties 408, 500, 505, 602, and 800 are Minhybrids; variety W240 is a Wisconsin hybrid; variety D4 is a Northrup, King and Co. hybrid.

[b] S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant.

races, but also differences in the resistance of corn varieties and teosinte. *Helminthosporium* Z and races 4 and 7 of *H. sativum* were almost as highly pathogenic as the virulent races of *H. carbonum* and *H. turcicum;* and races 1 and 5 of *H. sativum* were as virulent as *H. turcicum* race 1. Although teosinte plants were resistant to *Helminthosporium* Z, they were severely infected by *H. carbonum* race 1, which appears to be most closely related to *Helminthosporium* Z in many characters. These experiments prove that certain races of *H. sativum* and *Helminthosporium* Z may cause severe infection on corn in the field.

CONCLUSIONS

Helminthosporium turcicum, H. sativum, H. carbonum, and *Helminthosporium* Z cause diseases of corn in Minnesota. These Helminthosporia differed greatly in their virulence on 8 varieties of corn and one variety of teosinte. Races of *H. sativum* also differed greatly in pathogenicity and to some extent in symptoms produced on the foliage of corn. The identification of the casual agent by the symptoms alone would sometimes be difficult, because two or more Helminthosporia may attack the same leaf. In addition,

certain saprophytic fungi, at least under certain conditions, modify the type of lesion produced. Moreover, the symptoms vary to some extent with the variety of corn, the age of the plants, and the environmental conditions.

A large-spored *Helminthosporium* was isolated from diseased foliage of a corn plant. This organism was designated *Helminthosporium* Z until there was an opportunity to determine whether or not it was an undescribed species of *Helminthosporium* or a distinct variety or race of *H. carbonum*. *Helminthosporium* Z caused not only severe infection on the foliage of corn, but also stalk rot and root rot. In morphology it resembles somewhat *H. carbonum*, whereas the symptoms produced resemble more closely those produced by *H. sativum*. Although *Helminthosporium* Z may be a virulent pathogen on many varieties of corn, how prevalent and important it is in nature is not yet known.

Helminthosporium sativum is a virulent parasite on corn and teosinte. Although it has been known for a long time that this species comprises many parasitic races and that it has a very wide host range, it was not known that it was a common pathogen on corn.

The present studies have clearly shown that Helminthosporium diseases of corn constitute a complex problem and that there is need for much more work on the disease complex caused by *Helminthosporium* spp.

SUMMARY

Helminthosporium sativum was the most common pathogen isolated from diseased corn leaves in Minnesota. *H. carbonum*, *H. turcicum*, and *Helminthosporium* Z also were isolated and studied in considerable detail.

Helminthosporium sativum, not heretofore reported as pathogenic on corn, attacks it readily, although there are differences in pathogenicity of different races.

Helminthosporium Z resembles *H. carbonum* but differs in size of conidia and in pathogenicity on certain hosts, especially teosinte. It may be a new species of *Helminthosporium* or a variety or race of *H. carbonum*.

The host range differs for the several species of *Helminthosporium*. *H. sativum* has the widest host range. *Helminthosporium* Z infected Sudan grass in addition to corn, whereas it did not attack teosinte readily. *H. carbonum* infected teosinte severely; and *H. turcicum* infected sweet corn, teosinte, and Sudan grass. Varieties of corn and teosinte react differently to species and races of *Helminthosporium*.

Helminthosporium carbonum, *H. turcicum*, and *H. sativum* each comprise two or more parasitic races.

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FURTHER STUDIES OF THE BACTERIAL NECROSIS OF THE GIANT CACTUS[1]

ALICE M. BOYLE[2]

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The first report on the bacterial necrosis of the giant cactus (*Carnegiea gigantea* Britt. and Rose (*Cereus giganteus* Engelm.)) was published in 1942 by Lightle, Standring, and Brown (10). Since that year the toll taken by the disease has been great in the Saguaro National Monument, the Tucson mountain area, the Catalina Foothills, and in plantings on estates in the region where the giant cactus grows. Scenes such as shown in figure 1, A have become increasingly common. Thus, because of its economic as well as its botanical importance, studies of the disease have been continued.

REVIEW OF PREVIOUS WORK

The first symptom is a small, circular, light-colored spot, usually with a water-soaked margin, anywhere on the surface of the integument of trunk and branches (10). Parenchymatous tissues under the integument first appear water-soaked, but soon turn brown to almost black. As the infection progresses, the spots on the surface enlarge and become purplish. In some cases the integument may break and a brown liquid may exude. Slower decay of the tissues may go on without "bleeding," but the rotted tissues dry, break up into a granular, lumpy mass, and fall to the ground leaving the woody stelar strands bare.

Histologic and cytologic changes due to the bacterial invasion were discussed (10). Drying, cracking, and discoloration of the cuticle-covered epidermis and the hypoderma take place. The chlorenchyma loses its chlorophyll, becomes water-soaked, and later is discolored brown to black; all other parenchymatous tissues also appear water-soaked and later are discolored. Finally the soft tissues break down into a dark-colored mass. Cytologic changes leading to the breakdown of parenchyma tissues affect the entire cell. The wall loses its middle lamella by dissolution and eventually becomes entirely soft and irregular in outline. Early in the progress of the

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infection, the nuclear membrane thickens; the nucleoplasm loses its reticular aspect and becomes more or less homogeneous and retentive to stains; increase in bulk of the nucleus may be followed by shrinking, its outline becomes irregular and wrinkled, and disintegration follows.

Inoculation experiments were reported (10), showing a bacterium to be the cause of the giant cactus necrosis. Characteristics of the bacterium were given and the name *Erwinia carnegieana* Standring was proposed.

The disease is distributed over an area coinciding with the habitat of the giant cactus in the United States—an area 200 miles broad, north and south, by 250 miles long, east and west. The disease has been particularly destructive near Tucson, in Picacho Park, and near Maricopa, Arizona.

SPREAD OF THE DISEASE

Rapid spread of the bacterial necrosis of the giant cactus may be accounted for in part by infection of a healthy plant from a diseased one by way of closely associated root systems. The plants often grow very close together in groups. If one plant in such a group becomes diseased, almost invariably others of the group also succumb, lesions very often developing near the base. Over a period of years, one plant after another in the group is infected, so that eventually as many as ten or twelve giants in a cluster have been killed.

Erwinia carnegieana was isolated in 1944 from the exposed roots of a plant weakened by extensive basal lesions and blown over in a windstorm. Since that time several infected root systems have been observed and the roots cultured. This condition has been induced by inoculation (Fig. 5, C and D).

A "bleeding" cactus depositing exudate on the ground may be a source of infection to healthy cacti nearby. The exudate may consist of several gallons of almost black liquid which contains the bacteria that cause the disease. The exudate is often of sufficient quantity to soak the soil to a depth of 3 or 4 in. in a wide area about the diseased plant. Since many roots of the giant cactus are very close to the surface of the ground or partly exposed and have cortex broken by action of weather, rodents, or insects, infection of the roots of plants growing near an infected one is possible.

The bacterium *Erwinia carnegieana* is capable of living in the soil and there remaining viable for considerable time. Soil which was soaked with the exudate from a "bleeding" cactus was collected during the summer of 1945 and brought into the laboratory for study. Isolations were made and cultures of *E. carnegieana* obtained. The soil was then divided into two parts. One part was allowed to dry, and to the other was added enough water from time to time to keep it in about the same condition of moisture as when collected. At frequent intervals, varying from 5 days to a week, soil samples were taken from the two lots and isolations were made by the usual dilution method. After 46 days of exposure to Tucson's summer temperature and to drying in one case, *E. carnegieana* was isolated from both

lots of soil. The added moisture was found not to affect the length of time the bacterium remained alive in the soil, so it is not probable that summer rains would extend the life of *E. carnegieana* in the soil. Figure 1, C shows a cactus inoculated with the bacterium isolated from the soil 46 days after collection of the soil sample.

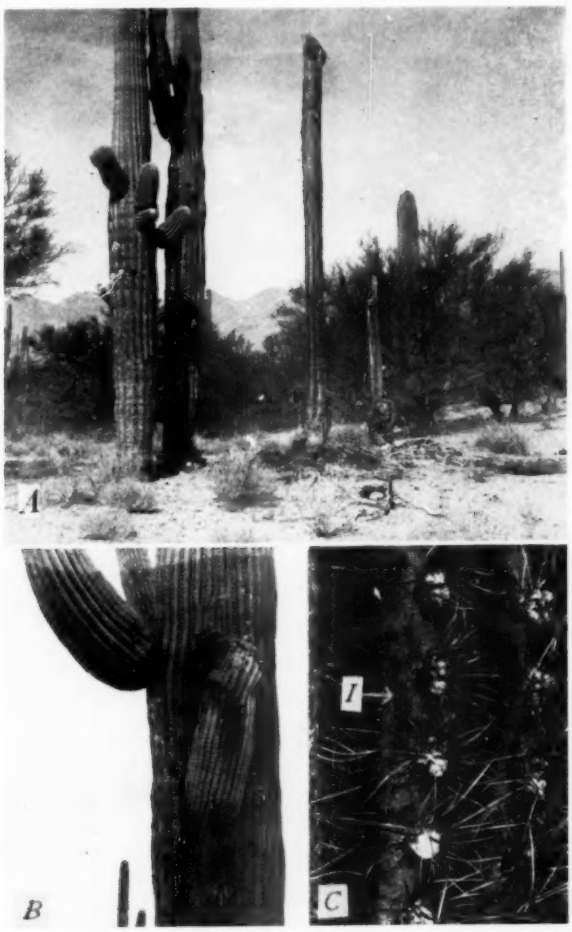

FIG. 1. A. Destruction of *Carnegiea gigantea* caused by *Erwinia carnegieana*, a frequently occurring sight in many giant cactus areas. B. Necrotic branch of giant cactus which caused infection of trunk at point of contact. C. Plant inoculated with *E. carnegieana* isolated from soil 46 days after collection. Inoculation point at *i*.

The two means of infection discussed may account to a great extent for the basal lesions that are so often fatal. Rather extensive lesions at the top of a giant cactus or on branches may not necessarily prove fatal to the plant. When a giant cactus is girdled at the base, however, it is so weakened that it easily breaks off in a windstorm. Such broken giants (Fig. 1. A) are a common sight in forests of giant cactus.

A plant or plant part may be infected directly in cases where a diseased branch comes in contact with a healthy trunk or branch. A diseased branch of giant cactus may become weakened and fall against a healthy part of the trunk of the same individual (Fig. 1, B) or even another growing near by. The movement of the diseased branch by the wind may injure the healthy tissues so that the parasite is able to enter the latter. In the case of the saguaro photographed, a lesion about 6 in. in diameter has developed on the trunk during the 8 months the branch has been dangling and striking against it in the wind.

By far the most important factor in the spread of the cactus necrosis, however, is the insect vector.

THE INSECT VECTOR

The opinion that an insect vector was chiefly responsible for the spread of the bacteria from diseased cacti to healthy ones was held by Lightle and co-workers (10). Tunnels, apparently made by insects, had been found associated with necrotic lesions. A study of the insects frequenting the giant cactus was undertaken by the present author. Because such a wealth of insect life is found in and on the plant (8, 9), many insects were collected, cultured, and reared, and their life histories studied in relation to the giant cactus before the vector was determined.

Prior to 1942, the giant cactus necrosis had been controlled (6) by removal and burial of badly diseased plants.

In the spring of 1942, the control work on the disease, which was being continued in the Saguaro National Monument by Dr. James Mielke, in the absence of Dr. Gill, afforded an opportunity for observation and study of the insects found in giant cactus. There was available a wealth of infected material with tunnels such as had been occasionally observed in the soft tissues of plants. The tunnels varied in direction and size. Careful dissection of tunneled plants showed "rot pockets" or lesions developing at random along the length of the tunnels, both internal and external to the stele of the plant (Fig. 2, A and D). *Erwinia carnegieana* was isolated repeatedly from tissue surrounding the tunnels.

In June 1942 the first larvae responsible for the tunneling were found in giant cactus (Fig. 2, C). When placed in a jar with fresh cactus tissue, larvae tunneled readily and rapidly directly through the cutinized epidermis and hypoderm as well as through the softer tissues (Fig. 2, B). *Erwinia carnegieana* was isolated from the surface and from the intestinal tracts of the larvae. In the latter case, specimens were surface-sterilized in 1 : 1000 mercuric chloride and crushed on agar plates.

Some of the larvae were successfully reared in wire cages in the laboratory, and the bacterium responsible for the necrosis of the giant cactus was isolated from the adult and from the surface of the eggs. In April, 1944, after inoculation experiments had shown the insect capable of transmitting the disease, adult specimens as well as larvae and pupae were sent to the

U. S. Bureau of Entomology where the insect was identified by Mr. Carl Heinrich as *Cactobrosis fernaldialis* (Hulst) (8).

Observation in the field and laboratory from 1942 to the present time have led to the conclusion that *Cactobrosis fernaldialis* has but one genera-

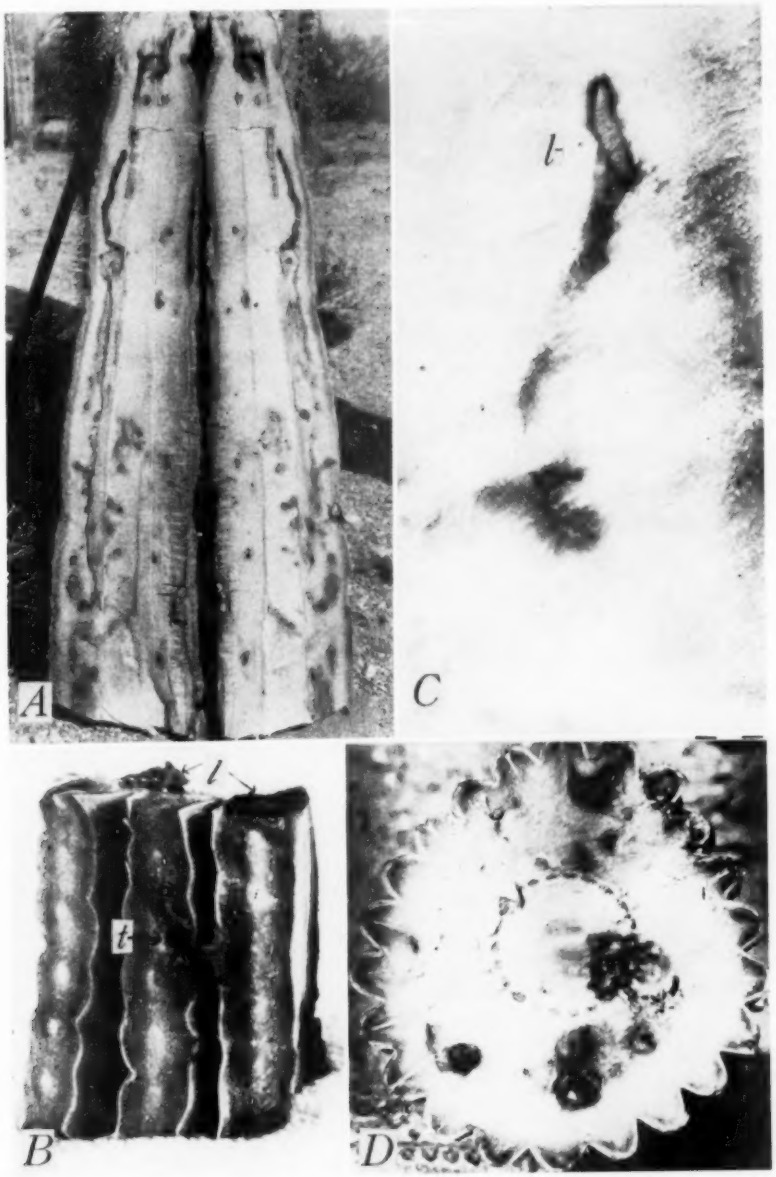

FIG. 2. A. Longitudinal section of branch of giant cactus showing tunnels made by larvae of *Cactobrosis fernaldialis*. B. Larvae of *C. fernaldialis* at *l* and tunnels made by them at *t*. C. Close-up view of *C. fernaldialis* (*l*) in a tunnel made by it in giant-cactus tissue. D. Cross section of branch showing tunnels made by larvae.

tion a year. The larvae have been found tunneling in giant cactus from November 2 until August 20, so it is apparent that the longest period of the insect's life span is its larval stage when it feeds in the giant cactus. The larvae are never found in tissues that are in a late stage of disintegration caused by *Erwinia carnegieana*, but rather constantly move away from such

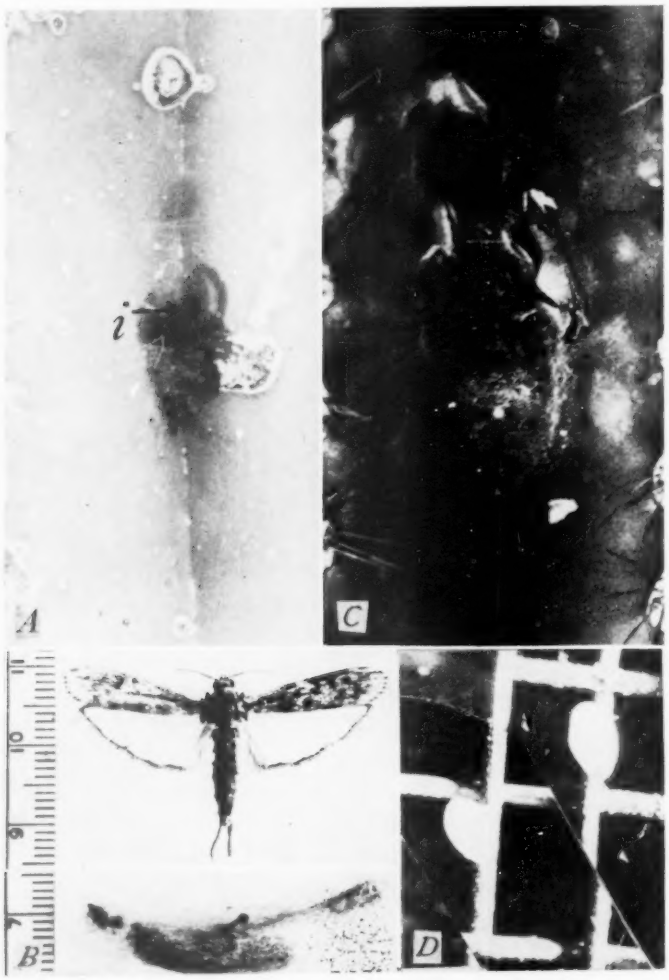

FIG. 3. A. Surface view of giant cactus showing emergence hole through which larva has left plant to pupate (*i*). B. *Cactobrosis fernaldialis* adult and pupa case containing pupa. C. Pupa of *C. fernaldialis* near emergence hole on surface of a giant cactus. Emergence hole lies in the shadow of the pupa. D. Eggs of *C. fernaldialis* on wire mesh of breeding cage.

areas as the disease advances into healthy tissue. From May through August, when larvae leave the plants to pupate, fresh, uncorked emergence holes are frequently seen (Fig. 3, A). Although the insects occasionally pupate on the plant near the emergence hole (Fig. 3, C), it is characteristic

of them to seek protection under fallen pieces of cactus, stones, or sticks. When the moths were reared in captivity the larvae never pupated in the open but always on the underside of available debris such as pieces of wood, cactus, or other objects.

The pupal stage is from 28 to 33 days' duration. The adult nocturnal moth, light tan with dark brown markings (Fig. 3, B), is short-lived, laying its eggs and dying within three days. This observation agrees with the report of Dr. Carl Heinrich on the life history of the insect (8). The eggs have been found in nature only twice, borne singly on a cactus spine near the apex of a low-hanging branch. In breeding cages they are seldom deposited on cactus plants placed in the cages but most often are deposited singly and in great numbers on the wire mesh of the cage (Fig. 3, D). During the 30-day period before hatching, the tiny eggs change color from their original cream through deepening shades of pink to a rather brilliant red. During the first few hours after the very small larvae emerge from the eggs, their mortality rate is high. They move around apparently aimlessly, but roughly about 10 per cent of the larvae reach a cactus plant, entering either directly through the unbroken cuticle and epidermis or, more often, by way of the softer tissues at the growing point.

Inoculations.—Since it was obviously impractical to build cages large enough to accommodate mature giant cacti, the plants used in these experiments were either very young ones grown from seed in the greenhouse and kept in cages $12 \times 12 \times 18$ in., or were plants 1 to 2 ft. high transplanted from the cactus forests to pots and placed outdoors in cages $4 \times 4 \times 5$ ft. The small, greenhouse-reared plants are not heavily cutinized, hence the $\frac{1}{8}$-in. larvae were capable of entering at any point. The larger cacti used were never entered except near the apex, and it is believed that in nature the larvae enter the plant through the soft tissues at the growing point. In dissected plants, both the larger ones mentioned above and those examined in the field, narrow tunnels near the trunk and branch tips appear to have been made by small larvae. As the distance from the plant apices increases, the tunnels widen, appearing to have been made by the larvae after they have fed and grown within the plant. The larger and therefore older steel-gray larvae may eat out a tunnel $\frac{3}{4}$ in. in diameter and many yards in length.

The period during which larvae are entering the cactus plants coincides with the 150-day period of growth at the trunk and branch apices reported by Macdougal (12). Within 10 days after larvae have entered plants, in a high percentage of cases, typical symptoms of the necrosis are apparent (Fig. 4, A and B).

Plants too large to place in the insect breeding cages also were inoculated with larvae. On January 27, 1944, eight larvae were placed in a cavity made by cutting out a plug about 3 in. in diameter at the epidermal end and extending, wedge-shaped, 5 in. into the plant. An inch was cut off the small end of the wedge, the larvae were inserted into the hole in the plant, the wedge was replaced and the cut sealed firmly with beeswax. Figure 5,

A shows the resulting lesion on February 24, 1944. "Bleeding" had started 3 days earlier, indicating that extensive breakdown of tissues had taken place, undoubtedly because of bacterial invasion since symptoms were identical with known symptoms of the disease in nature. By April 26, 1946, progress of the parasite had been arrested by the formation of layers of cork separating the lesion from healthy tissues (Fig. 5, B). A large cavity

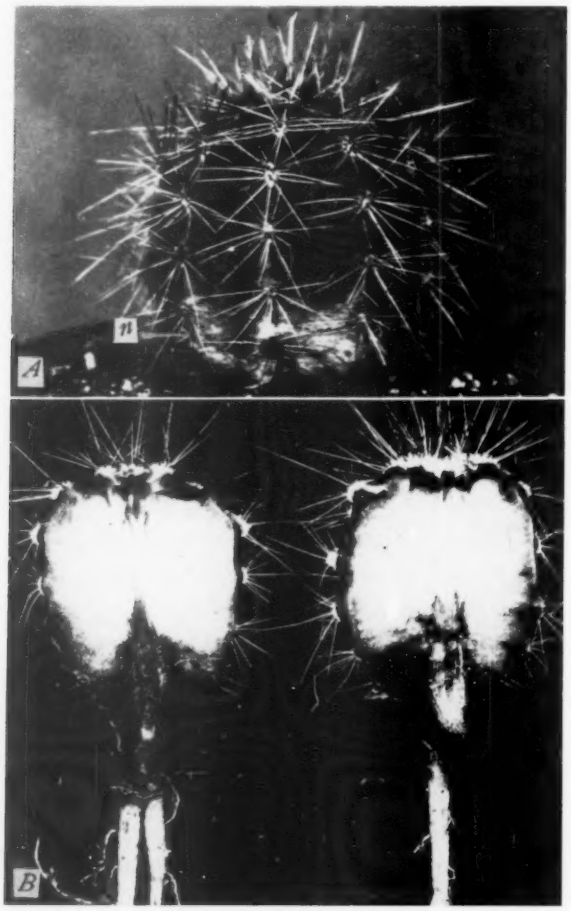

FIG. 4. A. Giant cactus 2 in. high showing necrosis at *n* as a result of invasion by larvae. B. The same plant sectioned to show tissue discolored due to necrosis.

extended almost to the woody cylinder. Whether the advance of the disease has been checked permanently is not yet certain. The plant is still under observation.

Another giant cactus was inoculated at the same time and in the same way, but at a point just above the ground line. The only noticeable symptom was a gradual loss of the normal dark green color of the entire plant, until the plant became pale yellowish-green. This condition has often been

observed in nature before any lesions, "bleeding," or other local symptoms appear. Such plants, after a long time, give evidence of the extensive necro-

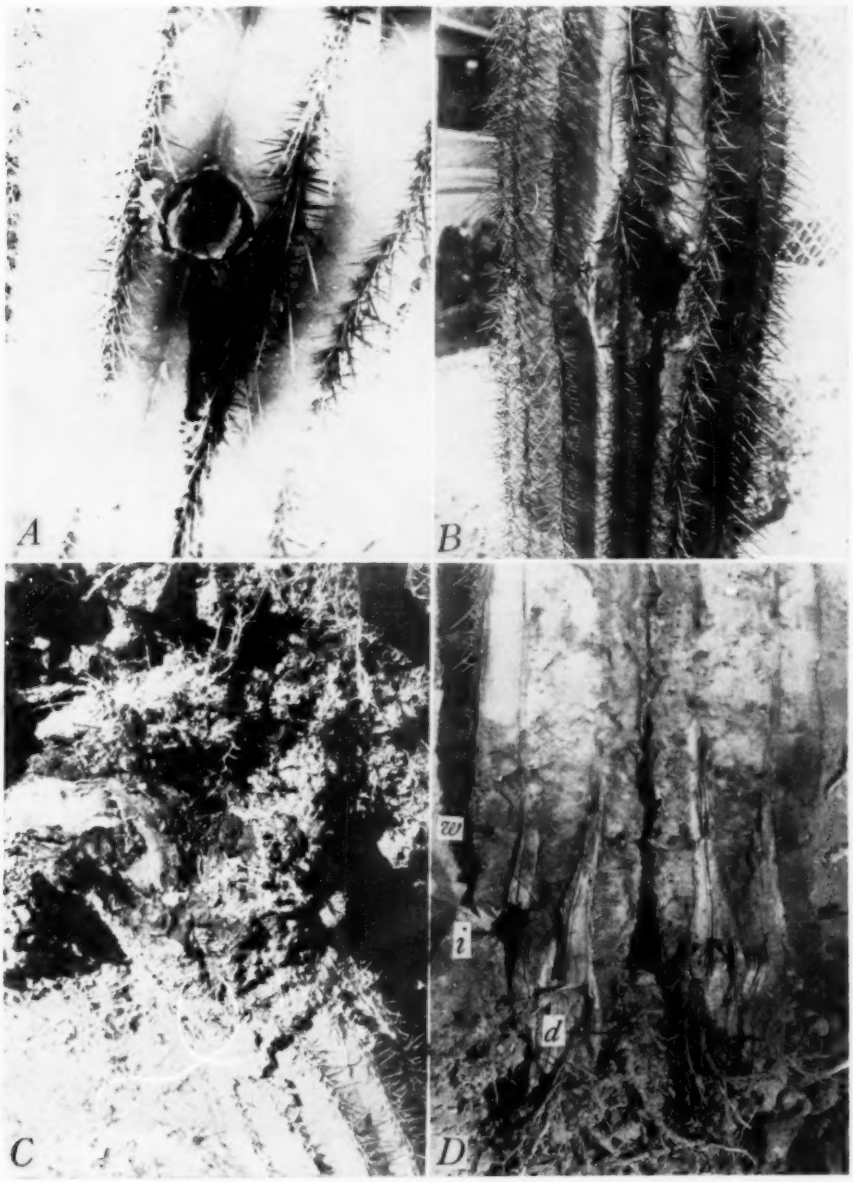

FIG. 5. A. "Bleeding" of lesion resulting from inoculation by larvae of *Cactobrosis fernaldialis*. B. Giant cactus shown in A, 2 years after inoculation: this plant received over twice the normal amount of water. C. Natural appearance of giant cactus killed by inoculation with larvae of *C. fernaldialis*. Base of plant with rotted root system and extensive basal lesion. D. Longitudinal section of giant cactus shown in C. Larvae of *C. fernaldialis* were inserted in healthy plant approximately at *i*; *w* shows infected, water-soaked tissue; *d*, decomposed tissue.

sis which has been progressing within them. And so it was with the inoculated plant under discussion. The plant was photographed March 12, 1948, a few days after it had blown over in the wind (Fig. 5, C). The root sys-

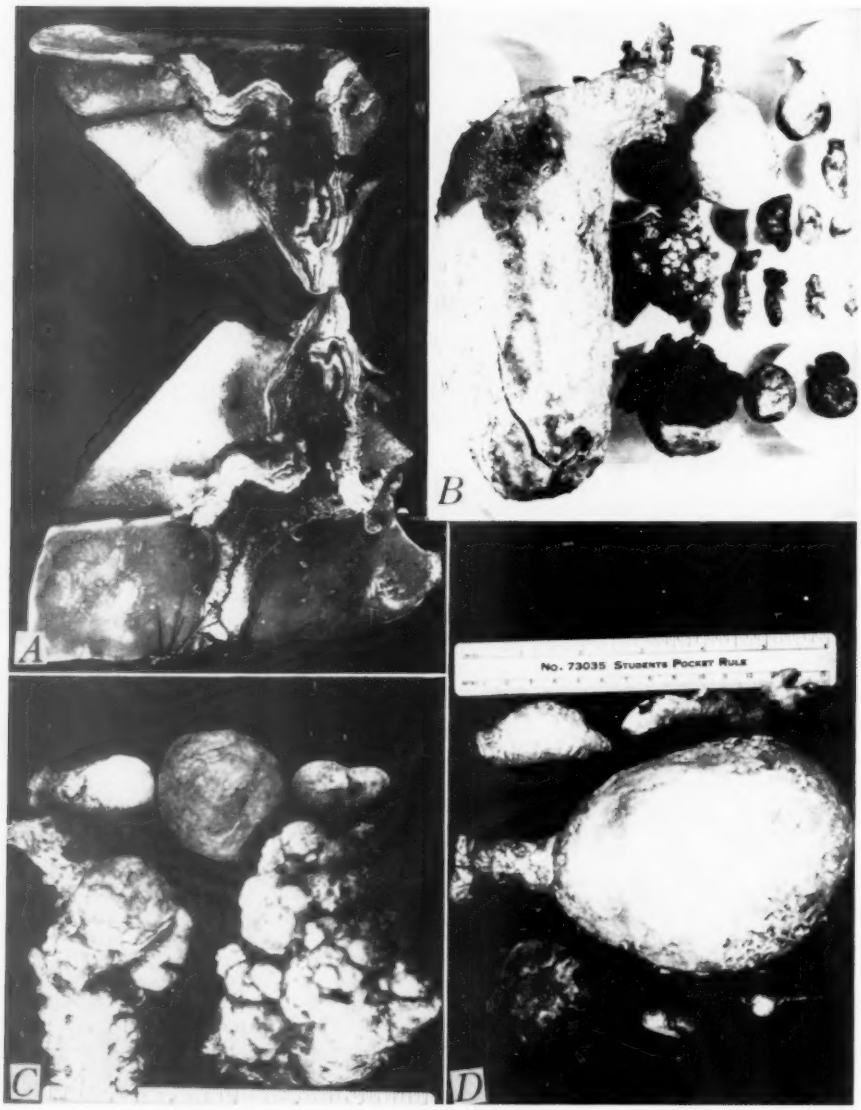

FIG. 6. A. Cone-shaped, suberized structure, split through the center to show layers of corky cells with strands of parenchyma cells between. B, C, and D. Cork-structures which developed around necrotic lesions in soft tissues of giant cactus.

tem was almost entirely rotted away, and the plant was girdled by a lesion at the base. In longitudinal section (Fig. 5, D) the entire base of the plant has the typical discoloration and softening.

Corking-off.—The "corking-off" process, which is one means of defense against invasion of the parasite, is common in nature as well as in experimentally inoculated plants. Formation of cork is stimulated as readily by any injury as by the invasion of the bacterium responsible for necrosis. Figure 6, A shows a cone-shaped, suberized structure enclosing an infected area, split open through the center to show layers of corky cells with strands of parenchyma cells. The white dots are crystals. The dark areas enclosed by the corky layers still contained living bacteria capable of progressing into the healthy tissues should breaks occur in the enclosing shell.

In many cases of natural infections and also those produced by inoculations, the corking-off does not keep pace with the rapid progress of the pathogen, or breaks through the cork layers occur and, finally, total destruction of the plant results. Peculiarly shaped cork structures are often found on the ground where a giant cactus has fallen and disintegrated, indicating that the cactus had repeatedly resisted the attack of the parasite by cutting off its progress temporarily by means of layers of suberized cells, and the parasite has just as often broken through these layers. Figures 6, B, C, and D, show that these cork structures may assume practically any size or shape, depending upon that of the lesion outlined. Tubular corked structures, the result of the corking-off of tunnels, are sometimes so extensive in length and branching and so thin-walled that they cannot be preserved sufficiently intact to be photographed.

Other Insects

Some of the insects other than *C. fernaldialis* found associated with necrotic lesions may play a part in the progress of the disease. Larvae of *Volucella* species are found often by the thousands lining a lesion on the surface between healthy and infected tissues. A flat, black beetle identified as *Hololepta yucateca* (Marseul), predacious on saprophagous insects, is usually found. A small black beetle and its larva are numerous, as is also a large larva of an unidentified moth. These larvae and adult insects have been found only in the disintegrated tissues of the plant, not progressing into normal tissues as does *Cactobrosis fernaldialis*. *Erwinia carnegieana* has been isolated from body surfaces but never from the intestinal tracts of any insect except *C. fernaldialis*. It is probable that some spread of the disease is accomplished by them, and their constant activity and presence in such large numbers may help to prevent the normal corking-off of the plant about a lesion, or may help cause breaks in the cork tissue facilitating the progress of bacterial invasion.

AGE OF THE GIANT CACTUS AND SUSCEPTIBILITY TO BACTERIAL NECROSIS

In 1942, when work was first started by the author, the number of old plants killed by the disease was noticeably so great that a relationship between age of plants and susceptibility to necrosis was considered a possibility. Since that time, so many naturally infected young plants have been found (Fig. 7) and inoculations have produced the disease in young plants

in such a high percentage of cases (Fig. 4, A and B), that this supposition has now been abandoned. The higher percentage of deaths of older plants can be adequately explained by the fact that they outnumber young ones. In some areas, notably the Saguaro National Monument, the percentage of

FIG. 7. A giant cactus 18 in. high showing a lesion resulting from natural infection with *Erwinia carnegieana*.

young plants is very small. It is difficult to find plants less than 10 ft. in height in this area. In the Tucson mountain area and in the Catalina Foothills, plants of all sizes are more plentiful but young ones are still very much in the minority.

The lack of young plants may be due to several factors. Flowers become infected with the rot bacterium and fail to produce seeds. *Erwinia carnegieana* has been isolated from partly rotted flowers and immature fruits collected from the ground where they had fallen. A similar condition has been produced experimentally by spraying blossoms with a bacterial suspension of *E. carnegieana*, and this bacterium has been reisolated from the rotted flowers. According to Benson and Thornber (2), and the writer's record of the flowering period of the giant cactus, this period coincides with that during which the young larvae of *Cactobrosis fernaldialis* are entering the plants. Although larvae have never been found in the partly rotted flowers and fruits collected, tiny holes thought to be made by the larvae have been observed.

This, then, accounts for a reduction in seed production. Nevertheless, a high percentage of flowers do produce mature fruits. As a result of overgrazing, ground cover in many of the cactus areas has been so greatly reduced that no protection is offered the fruits when they drop to the ground and many are consumed by rodents or birds. Some escape this fate to germinate and produce small plants which, unprotected, are also eaten by rodents. The result is a very few young plants which happen to start life in a clump of mesquite or other desert shrub.

H-ION CONCENTRATION OF CELL SAP OF GIANT CACTUS AND ITS RELATION TO BACTERIAL NECROSIS

Changes in H-ion concentration of the sap of plant tissues as a result of disease are expected. Fischer and Gaümann (4, pp. 368–371) give an excellent review of the literature on this subject. Examples are given to show changes in pH toward the acid side with infection by one parasite, either bacterial or fungus, in a certain host, and a change toward the alkaline side with a second parasite in the same host. Also the same parasite may cause changes in tissue liquids of two different hosts, in one case toward the acid side and in the other toward the alkaline. The conclusion, then, would be that such changes in pH of plant tissues due to invasion by a parasite are the sum of the reaction of the host to the parasite and of substances given off into the plant by the parasite.

Gaümann (5, pp. 322–323) discusses the fact that pH is often a limiting factor in invasion of plant tissues by a parasite.

Using a Beckman pH meter in the field, pH readings were taken on cactus tissues at different seasons of the year. Pieces of plant tissues to be tested were removed from the plant, the sap pressed out with a small hand press, and the test made immediately. The following results were obtained: apparently uninfected plants, pH 5 to 5.5; healthy-appearing tissue of infected plants, 7 to 7.4; discolored tissue not yet broken down, 8.7 to 9; soft, broken-down tissues, 3 to 3.5.

The readings for healthy tissues agree with those of Macdougal (12). The difference between the pH of uninfected plants and healthy-appearing

infected plants was first thought to be due to a genetic difference in the plants, making the latter more susceptible to the disease. *Erwinia carnegieana* was tested in broth cultures adjusted to pH from 3.0 to 8.0. A pH of 7 to 7.2 is optimum for its growth, and very little growth takes place at pH 5 to 5.2 and none below pH 5. However, pH readings taken on noninfected 5- and 6-year-old plants grown in the greenhouse from seed were in exactly the same range as that of older plants. Dr. Macdougal's work (12) has shown no differences in pH of giant cactus tissues due to age. Since the plants tested were grown from seed of infected as well as healthy plants, it is probable that a difference in pH would have been apparent had any genetic difference been present. No attempt has been made to explain changes in pH of the infected tissues since chemical analyses have not been made.

RAINFALL AND BACTERIAL NECROSIS OF THE GIANT CACTUS

In an effort to determine whether amount of rainfall influences the progress of giant cactus necrosis, eleven healthy giant cacti were transplanted in April 1944 to the University of Arizona campus. These were used for inoculation work and experiments concerning water relations.

TABLE 1.—*Monthly additions of water to five of eleven[a] giant cactus plants studied at Tucson, Arizona, in 1945*

Month	Average rainfall[b] 1875–1944 (inches)	Rainfall 1945 (inches)	Additions of water, in inches of rainfall				
			To attain average		Excesses		
			Plant No.		Plant No.		
			5	9	3	7	11
January	0.42	0.65	0.00	0.00	0.42	1.40	0.42
February	0.94	0.41	0.53	0.53	1.47	2.00	1.47
March	0.77	0.62	0.15	0.15	0.92	2.01	0.92
April	0.35	0.08	0.27	0.27	0.97	1.00	0.97
May	0.19	0.00	0.19	0.19	1.90	2.90	1.90
June	0.27	0.00	0.27	0.27	1.35	2.40	1.35
July	2.16	1.22	1.14	1.14	2.16	2.50	2.16
August	2.29	2.95	0.00	0.00	2.29	2.52	2.29
September	1.19	0.12	1.05	1.05	1.26	1.50	1.26
October	0.26	1.42	0.14	0.14	1.42	1.50	1.42
November	0.80	0.00	0.80	0.80	1.60	2.00	1.60
December	1.07	0.16	0.91	0.91	1.98	2.00	1.98
Total	11.41	7.63	4.73	4.73	17.74	23.73	17.74

[a] No additions to plants Nos. 1, 2, 4, 6, 8, and 10.

[b] Rainfall data for comparison, obtained from weather station located approximately 200 ft. from row of giant cacti studied.

As will be seen by referring to table 1, the average rainfall for the years 1875 to 1944 was taken for a basis. Plants 2, 4, 6, 8, and 10 were given no additional water although the total rainfall for the year 1945, during which the tests were run, was below the average. Plants 3 and 11 were given 17.74 in. water additional to the rainfall for the year, or a total of 25.37 in., more

than twice the average rainfall. Plant 7 was given 23.74 in. water in addition to the rainfall, or a total of 31.37 in., nearly three times the average. Plants 5 and 9 were given just enough additional water to bring the total amount up to the average each month. Actually the total amount of water received by these giants was slightly in excess of the average because, although the total rainfall for 1945 was below average, that for the months of January, August, and October was slightly above.

After determining the amount of water in cubic feet to be added to the 6×6-ft. depressed areas around the giant cactus, a water meter was used to measure the additional water applied. At the end of each month when the figures on total rainfall for the month became available, the treatment was given.

The giant cacti were inoculated by different means—by the stab method, by larvae, and by spraying the flowers with a suspension of *Erwinia carnegieana*. No difference in speed of infection or progress of the disease was noted.

Plant 11 (Fig. 5, A and B) received more than twice the average normal amount of water; plant 1 (Fig. 5, C and D) received no extra water. Floral infection in plants 4 and 7 took place in about the same percentage of cases as did infection by the stab method in plants 3 and 9. Conclusions based upon results with so few plants cannot be final, but results are supported by later observations made in the field. The total rainfall for 1944 was 13.32 in., and for 1946, 13.68 in.; both above the long-time average. The year 1947 totaled only 5.72 in. There has been no substantial difference in the progress of the disease during these years, which seems to substantiate the conclusion, obtained experimentally, that amount of rainfall is not a contributing factor in the progress of the disease.

STUDIES WITH THE PATHOGEN

Cultural Studies

The bacterial cultures used in these comparative studies originated from fresh isolates that had been tested for pathogenicity. The tests were run at 30-day intervals over a 15-month period. Stock cultures were kept in the refrigerator under sterile mineral oil. Each month transfers were made to nutrient broth, and after 24 hr. were utilized as inoculum for all the media employed in the tests. Transfers were also made from the stock cultures to potato-dextrose-agar slants, incubated until good growth was evident and placed under sterile mineral oil in the refrigerator until the next series of tests was run about a month later. The Manual of the Society of American Bacteriologists (13) was followed in these tests.

Durham fermentation tubes were used for all fermentation studies. Bacto-nutrient broth with 1 per cent carbohydrate added and brom cresol purple as an indicator were used throughout.

All plant-tissue agars were made by using 15 gm. agar, 20 gm. dextrose,

and 200 gm. plant material to the liter. The plant material was cooked in about 500 cc. of water until soft, the water drained off, and enough tap water added to bring the amount of liquid to 1 liter.

The steamed sweet potato, rice, and cornmeal were prepared by cooking the materials enough so that they could be smoothly spread in the bottom of a Petri dish to a depth of about $\frac{1}{4}$ in.; adding a small amount of water, and sterilizing in the autoclave for 20 min.

Other media were prepared according to the Manual of the Society of American Bacteriologists, except that spirit-blue agar which was used for tests of lipolytic activity was prepared according to Starr and Burkholder (17, 18).

Tests for indole and for nitrate reduction were carried out each time a series of cultures was run.

Gram stains and inoculations to determine possible changes in pathogenicity of the bacteria were also made. The necrosis bacterium was compared with four isolates of *Erwinia carotovora:* No. 1 from onion, No. 2 from garlic, No. 3 from spinach, and No. 4 from carrot.

Tables 2 and 3 show the results of the work at the beginning of the tests in May, 1943. At this time inoculations showed all the cultures to be strongly pathogenic, *Erwinia carnegieana* was Gram positive, and all the *E. carotovora* strains were Gram negative.

Tables 2 and 3 show the physiological and cultural changes resulting during the period from May to December, 1943, when transfer had been made and cultures run each month. By comparison it will be noted that *Erwinia carnegieana* and *E. carotovora* isolate No. 1 underwent comparatively few changes. Isolate No. 2, almost identical with *E. carnegieana* in May, still resembles it markedly. *E. carotovora* No. 4 changed very little during this period; the greatest number of changes occurred in isolate No. 3. No apparent decrease in pathogenicity of any of the bacteria could be detected up to this time.

Between December, 1943, and August, 1944 (Tables 2 and 3), a gradual decrease in vigor of growth in and on all media is noticeable. Most strains of *Erwinia carotovora*, but no known strains of *E. carnegieana*, lost the ability to utilize carbohydrates; exceptions were some of the di- and monosaccharides. Pathogenicity was completely lost. *E. carnegieana* by this time retained gentian violet only slightly in Gram staining, so was more Gram negative than positive, while *E. carotovora* No. 2 became Gram positive.

Erwinia carotovora isolate No. 1 did not originally produce indole but acquired the ability by December, 1943. By the end of the testing period, indole was again not produced; isolate No. 2 lost its ability to produce indole and did not regain it. In this respect it differs from *E. carnegieana* which it closely resembled in May, 1947. *E. carnegieana* retained its indole-producing ability throughout the tests; isolate No. 4 lost and regained the

TABLE 2.—*Comparison of reactions[a] of Erwinia carnegieana and E. carotovora with various carbon sources in May, 1943, December, 1943, and August, 1944*

Carbon source	*E. carnegieana*			Isolates of *E. carotovora*											
				1			2			3			4		
	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.
Monosaccharides															
Arabinose	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	A + G	A + G	A + G	A + G
Dextrose	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G
Fructose	A + G	A + G	A + G	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	A + G	A + G	A + G	Alk
Galactose	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	A + G	Alk
Levulose	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	Alk	Alk
L. xylose	A	A	Alk	Alk	Alk	Alk	A + G	A	Alk	Alk	Alk	Alk	A + G	Alk	Alk
Rhamnose	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	A + G	A + G	A + G	Alk
Xylose	A	A	Alk	A + G	A + G	Alk	A + G	A	Alk	A + G	Alk	Alk	A + G	A	Alk
Disaccharides															
Lactose	Alk	Alk	Alk	A + G	A + G	Alk	Alk	Alk	Alk	A + G	A + G	A + G	Alk	Alk	A + G
Maltose	A + G	A + G	A + G	A + G	A + G	Alk	Alk	Alk	A + G	A + G	A + G	A + G	Alk	Alk	Alk
Melibiose	A + G	Alk	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	Alk	Alk	A + G	A + G	Alk
Sucrose	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G
Trisaccharides															
Melezitose	Alk	Alk	Alk	A	A	Alk	A	Alk	Alk	A	Alk	Alk	A	A	Alk
Raffinose	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	A + G	Alk
Polysaccharides															
Glycogen	A + G	A + G	Alk	A + G	A + G	Alk	A + G	A + G	A + G	A + G	A + G	A + G	A + G	A + G	Alk
Inulin	Alk	Alk	Alk	A	A	Alk	A + G	Alk	Alk	A	Alk	Alk	A + G	A + G	Alk
Alcohols															
d-Sorbitol	A + G	Alk	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	A	A	A + G	A + G	Alk
Dulcitol	Alk	Alk	Alk	A + G	A + G	Alk	Alk	Alk	Alk	A + G	A + G	Alk	Alk	Alk	Alk
Inositol	A + G	Alk	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	Alk	Alk	A + G	A + G	Alk
Mannitol	A + G	Alk	Alk	A + G	A + G	Alk	A + G	Alk	Alk	A + G	Alk	Alk	A + G	A + G	Alk
Glucosides															
Aesculin	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk
Salicin	A + G	A + G	Alk	Alk	Alk	Alk	A + G	A + G	Alk	Alk	Alk	Alk	A + G	A + G	Alk

[a] Alk = alkaline; A = acid; G = gas.

TABLE 3.—*Growth*[a] *of Erwinia carnegieana and four isolates of E. carotovora on media May, 1943, December, 1943, and August, 1944*

Medium	*E. carnegieana*			Isolates of *E. carotovora*											
				1			2			3			4		
	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.
Fermi's solution	Sl	N	N	N	N	N	Sl	Sl	N	N	N	N	N	N	N
Cohn's solution	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Endo's agar	A, MS	A, MS	A, MS	A, MS	A, MS	F, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	F, MS
Malt extract agar	A	A	F	F	F	F	A	F	F	A	F	F	A	A	F
Simmons' citrate agar	Sl	N	N	N	N	N	Sl	N	N	Sl	Sl	Sl	F	F	F
Giant cactus agar	A	A	F	Sl	Sl	F	Sl	Sl	Sl	F	F	Sl	F	F	Sl
Asparagus agar	A	A	F	A	F	F	F	A	F	A	A	F	A	A	F
Carrot agar	A	A	F	F	F	F	A	A	F	F	F	F	A	A	F
Spinach agar	F	F	F	F	F	Sl	F	F	F	A	A	F	F	F	F
String-bean agar	F	F	F	F	F	Sl	F	F	F	F	F	F	F	F	F
Sweet-potato agar	A	F	F	A	A	F	A	A	F	A	A	F	A	F	F
Steamed sweet potato	A	A	F	A	A	F	A	A	F	A	A	F	A	A	F
Steamed rice	A	A	F	A	A	F	A	A	F	A	A	F	A	A	F
Steamed cornmeal	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F

[a] Sl = slight; F = fair; A = abundant; N = none; MS = metallic sheen.

ability to produce indole and lost its nitrate-reducing ability entirely. This ability was retained by all other strains of *E. carotovora* and by *E. carnegieana*. No hemolytic action or lipolytic activity was shown by any of the organisms throughout the test.

The evidence presented as a result of these tests indicates that *Erwinia carnegieana* belongs to the soft-rot group of bacterial plant pathogens. The group is an extremely variable one, and consequently results of cultural and physiological tests depend upon age of the culture and, in the case of strains of *E. carotovora*, the host from which the bacterium was isolated.

Agglutination Tests

Immunologists in the field of animal pathology have used plant materials to study the characteristics of plant proteins as antigens (21). The first use of the technique in the botanical field was for the purpose of detecting adulteration in flours and meals and foreign seeds in seed stocks. Later these methods were applied to genetic studies in separating and identifying strains and varieties of agricultural plants (23). The technique was applied to such mycological problems as the differentiation of varieties of yeasts and determination of relationship of certain fungi.

The first extensive piece of work dealing with a phytopathological problem was done by a soil bacteriologist interested in the bacteria of the nodules of legumes (24). The serological characteristics of strains were found to be definite, and to correlate with cross-inoculation of host plants. Stevens (19) made a careful study of 55 strains of nodule bacteria representing seven groups of cultivated legumes. His results confirm the earlier work as to the serological properties of the bacteria. Wright (22) found that the nodule bacteria of the soybean are distinct serologically, that serological characters are constant, and that they are unchanged by continued artificial culture or by plant passage.

Nelson (14) applied the agglutination test to a phytopathological problem. He found that he was able, by agglutination tests, to distinguish between the proteins of a flax resistant to wilt caused by *Fusarium lini* (*F. oxysporum* var. *lini*) and a variety which was susceptible. Paine and Lacey (15) applied the agglutination test to a study of *Bacillus lathyri*, *Bacterium phaseoli*, and *Aplanobacter michiganense*. Although the first two gave group agglutination, the workers concluded that they could be differentiated by this method. *Aplanobacter michiganense* failed to agglutinate with serum of either of the other two. Takimoto (20) reported that serological tests indicate that the bacteria isolated from soft-rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others in culture. Goldsworthy (7) reported the formation of specific agglutinins, with a titre of 1 : 10,000 in rabbits following intravenous injections with *Bacterium maculicolum*, a cauliflower pathogen. He used these agglutinins to determine the presence of the bacterium in soils. Link and

Sharp (11) found that *Bacterium*[3] *campestre*, the crucifer pathogen, although distinct, is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense* and less closely to *Bact. flaccumfaciens*. Their findings also show that biological specificity shown in their host relations is correlated with serological specificity. A. R. Stanley (16) reported on a study by the use of agglutination tests, of 120 cultures of soft-rot and colon bacteria. He reported that, although individuals of this large group were physiologically identical, some showed only a moderate agglutination, others showed no agglutination at all, and still others were thought to be identical serologically. The author also concluded that the soft-rot bacteria are closely related serologically to the colon-typhoid-dysentery group of bacteria. An extensive bibliography is given dealing with the use of serological methods in phytopathological work.

The object in using agglutination tests in the work with *Erwinia carnegieana* was to try to determine relationships between *E. carnegieana* and the strains of *E. carotovora* used in the cultural work described above. All freshly isolated bacteria were used in cultures which were tested for pathogenicity. The procedure followed was that given in the Manual of the Society of American Bacteriologists (13). A 24-hr.-old culture of *E. carnegieana* was injected intravenously into a rabbit, 2.5 cc. twice a week for 6 weeks. The serum obtained from the rabbit was employed in agglutination tests that were set up with the homologous strain and the four *E. carotovora* strains as antigen. The serum agglutinated the homologous strain completely at 1:10,000 and markedly at 1:20,000. No agglutination took place with any of the four *E. carotovora* strains as antigen. The work indicates that there is no antigenic relationship between *E. carnegieana* and the *E. carotovora* strains used. The use of a large number of soft-rot strains comparable to the number reported by Stanley in the paper quoted above might have shown relationships of "H" or "O" antigens. Antigenic analysis such as has been carried out with *Salmonella* group of bacteria might be of value in working out relationships in the soft-rot group.

Thermal Death Point

One marked difference between *Erwinia carnegieana* and *E. carotovora* is their thermal death points, found by the writer to be 59° C. in the case of the former, or 7° higher than that reported for *E. carotovora* (3).

CONTROL

Before the insect vector and its life history were known, control of the disease was attempted simply by opening up and cleaning out lesions which were recognizable in the plant by purplish-black areas on the integument. Cork was then quickly formed about the cavity, and although an unsightly hole resulted, this simple method was effective unless the plants were so tun-

[3] The genus *Bacterium* is now changed to *Xanthomonas*.

neled by larvae that numerous new diseased spots appeared later. Badly diseased plants were cut down and destroyed in an effort to reduce the source of infection or at least the amount of inoculum (6).

As knowledge of *Cactobrosis fernaldialis* and its habits has grown, control of the insect has come to be considered the most promising means of control of the disease. Two per cent D.D.T. is not effective against adults or larvae of the moth. Tests were made by spraying insects kept in breeding cages.

Erwinia carnegieana was tested by the plate-penicylinder method (1), using crude penicillin made in the laboratory of the Department of Plant Pathology. It was found that penicillin had an inhibitory effect on *E. carnegieana* (Fig. 8, A) but none on *E. carotovora*. When commercial penicillin became available, the tests were repeated with the same results.

Although the use of penicillin in control work is not practical in large-scale operations, its application has been found effective in cases in which only a few plants are involved. The treatment does result often in a cavity, but not so unsightly a one as that left by digging out the decayed tissue. The results so far with plants treated by injecting penicillin into a lesion with a hypodermic needle indicate that the penicillin is able to diffuse through the plant tissues for a considerable distance. The plant pictured in figure 8, B showed at the time of treatment an unruptured purplish-black area about 4 in. in diameter at *i*. Almost a quart of crude penicillin was put into the lesion by plunging the hypodermic needle through the middle of the necrotic spot. As is often the case, the involvement of plant tissues was more extensive than appeared on the surface. Ten days after treatment the lesion had opened and was "bleeding" freely. Two weeks later the photograph was taken. No noticeable change took place in the ensuing three months. It is the opinion of the author that the holes above the site of treatment mark the site of another lesion present at the time of treatment but not yet apparent, and that diffusing of penicillin through the plant was sufficient to kill the bacteria present and check the progress of the disease. In order to come to a conclusion it would be necessary, of course, to collect data on treatment of a greater number of plants.

When lesions are detected and treated while still small, results are as seen in figure 8, C. The cactus illustrated was treated and photographed at the same time as the one shown in figure 8, B and has remained unchanged in appearance for the last three months. Of the twenty plants treated with penicillin, all except one show results similar to those photographed, varying in appearance with the extent of the lesion treated. The one exception was a plant with a discolored spot only 2 in. in diameter on the cutinized epidermis. Two weeks after treatment there was very little left of the plant but the woody stelar strands. Evidently involvement of tissues was extensive before the treatment was given.

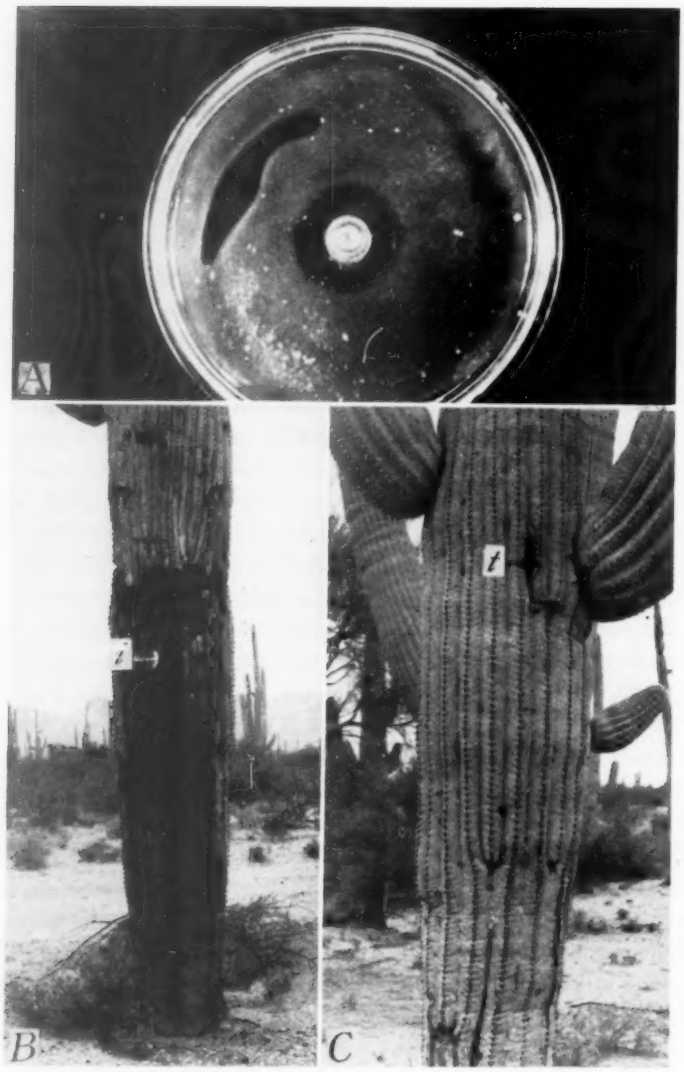

FIG. 8. A. Effect of penicillin on *Erwinia carnegieana*. Antibiotic has diffused outward from cylinder and killed bacteria in the surrounding circular area. B. Giant cactus 24 days after treatment with penicillin: point of inoculation at *i*. Diffusion of penicillin evidently stopped bacterial action in infected tissues above point of inoculation. C. Small necrotic lesion treated with penicillin at *t*. Photographed 24 days after treatment.

SUMMARY

The previous work on the bacterial necrosis of the giant cactus is reviewed, and the rapid progress of the disease and its importance are emphasized.

Three possible methods of spread of the disease are discussed: contact of the root system of one infected plant with the roots of other individuals

in a group; saturation of the ground around an infected plant, thus furnishing inoculum for the spread of the disease to other plants through wounds in the roots; and by the insect vector, *Cactobrosis fernaldialis*, the most important factor concerned with the rapid progress of bacterial necrosis in the giant cactus forests of Arizona.

Certain factors and their relation to susceptibility to the disease and its prevalence are discussed. The possibility that there is a correlation between age, or between amount of rainfall, and mortality of giant cactus plants is not accepted. The role of insects other than *Cactobrosis fernaldialis* found in necrotic lesions is considered.

Studies concerning *Erwinia carnegieana*, the bacterium which causes the necrosis of the giant cactus, are reported. The result of cultural studies shows that *E. carnegieana* belongs to the soft-rot group on the bases of physiological and cultural differences. Agglutination tests show no antigenic relationship, however, between *E. carnegieana* and the four isolates of *E. carotovora* used in the tests. In addition to this difference, *E. carnegieana* is inhibited by penicillin and has a thermal death point 7° above that reported for *E. carotovora*.

Reference is made to the preliminary measures for the control of necrosis. Determination of the vector of the causal parasite and its life history should afford a scientific basis for more effective measures.

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PHYTOPATHOLOGICAL NOTES

Corticium Crown Rot of Gazania.—Gazania rigens, a common ornamental in California, is usually grown to serve as ground cover or as a border plant. Under conditions of high temperature, entire plantings or portions of plantings are killed by crown rot.

In 1948 almost all of the plants which were growing in flats in a nursery in Stanislaus County were severely affected by crown rot. The original stock was obtained by the nursery from a propagator whose stock was also

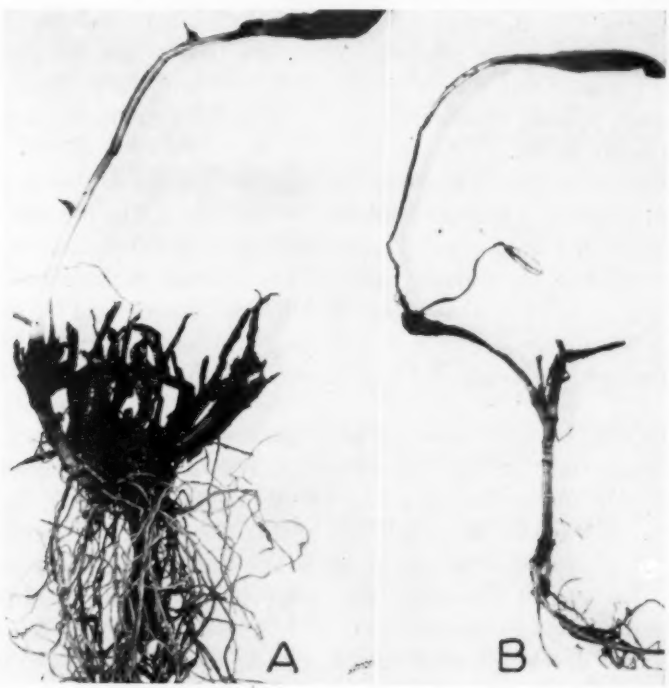

FIG. 1. Corticium crown rot of *Gazania.* A. A severely diseased *Gazania rigens* plant grown in the greenhouse. B. Cankers produced by *Corticium solani* on one of the shoots removed from the crown.

found to have the disease. The disease was also observed in three outdoor plantings in Alameda County.

The infection occurs at or below the ground line. The symptoms on the above-ground parts appear as wilting and withering of the leaves, beginning at the base and sometimes accompanied by twisting. The dead leaves and petioles become limp, fall to the ground, and easily become detached so that the diseased crown appears as a dark-brown to black mass of dead stems (Fig. 1, A and B).

Platings were made on acidulated potato-dextrose agar from diseased petioles and stems. After fungus mycelium could be seen under a binocular dissecting microscope, the tips of hyphae were transferred onto potato-dextrose slants and pure cultures were thus established. The fungus formed a sparse growth and produced sclerotia in culture in 2 to 3 weeks. Through the kindness of Dr. Byron Houston it was identified as *Corticium solani*. The optimum growth temperature for this strain was between 28° C. and 34° C. No growth was observed at 40° C.

For pathogenicity tests, the fungus was grown in Czapek's culture solution and only the mycelial mat was used in the tests. The mat was broken into small pieces in a Waring Blendor and poured over the surface of pasteurized soil (or mixed with it) in 6-in. pots in which healthy plants of *Gazania rigens* were growing. The plants inoculated with the fungus and kept outdoors during cool portions of the year showed no symptoms of the disease; but when these plants were transferred into the greenhouse at 28° C. and higher, wilting began after one week and the plants were completely destroyed in six weeks.

The relation of temperature to the fungus in culture closely paralleled that in soil either in the greenhouse or in the field. The disease has never been observed on plants grown under cool conditions, but is known to have been destructive in the warmer areas of the interior of the State.

Inoculations showed that *Gazania uniflora* and *G. splendens* are susceptible to the disease.—PETER A. ARK and ROBERT S. DICKEY, Division of Plant Pathology, University of California, Berkeley, California.

Botrytis Leaf and Blossom Blight of Saintpaulia.[1] The greenhouse culture of African violet (*Saintpaulia ionantha* Wendl.) has become an industry of considerable importance because of the demand for this flower as a houseplant. A leaf and blossom blight of African violet has occurred in the floricultural greenhouses at Michigan State College for some time. The symptoms, the causal organism, and suggestions for the control of this disease are discussed in this note.

Though no literature reference has been found of the occurrence of *Botrytis cinerea* Pers. on African violet, this fungus has been reported on such greenhouse-grown ornamentals as chrysanthemum and poinsettia,[2] geranium,[3] and cyclamen,[4] and on many ornamentals grown outdoors such

[1] Thanks are due to Evan Roberts, Supervisor of the Floriculture Greenhouses, for the Saintpaulia plants used in the course of these investigations and for supplying the initial infected Saintpaulia tissue, and to Clyde A. Bryant, Sr., Department of Horticulture, for aid in the original isolations and cross-inoculations.

[2] Spaulding, P. Botrytis as a parasite upon chrysanthemums and poinsettias. Mo. Bot. Gard. Ann. Rept., pp. 185–188. 1910.

[3] Melchers, L. E. Botrytis blossom blight and leaf spot of geranium and its relation to the gray mold of head lettuce. Jour. Agr. Res. [U.S.] **32**:883–894. 1926.

[4] Hansen, H. N., and C. M. Tompkins. Cyclamen petal spot, caused by Botrytis cinerea, and its control. Phytopath. **38**: 114–117. 1948.

as dahlia,[5] lupine,[6] rose,[7] and aster.[8] *Botrytis* has also been reported on a member of the Gesneriaceae by Baker,[9] who states that it causes a bud rot of *Sinningia*.

The disease usually first appears on *Saintpaulia* on the under side of the leaf petioles in the proximity of the pot rim. The first symptom is a small watersoaked lesion which may enlarge rapidly, circumscribing and extending lengthwise in the petiole, and on through the leaf blade. The necrotic area becomes brown to black with age.

The blossom-blight phase of this disease, which often originates in the throat of the corolla, produces premature fading and a watersoaked appearance of the corolla. If certain environmental conditions exist, *i.e.*, low light intensity, high humidity, and poor air circulation, the fungus may sporulate profusely on the infected tissue.

Infected corollas may drop on healthy leaves and thereby serve as a source of inoculum for the leaves. The disease has also been observed to spread from leaf to leaf where they are in direct contact with each other.

Isolations from diseased *Saintpaulia* tissue were made on potato-dextrose agar and have consistently yielded a fungus which has been identified as *Botrytis cinerea* Pers.[10]

The fungus produced conidiophores and conidia abundantly on oatmeal agar and potato-dextrose agar. Some conidia were produced on Czapek's medium, but very few on cornmeal agar.

Isolates of the fungus were pathogenic to *Saintpaulia ionantha* (Wendl.) and various named varieties such as "Blue Boy," "White Lady," "Blue Girl," "Plum," and "Pink Delight." The inoculum used was prepared by growing the fungus on potato-dextrose agar in Petri plates. After conidia were formed, sterile water was added and the spore suspension obtained was sprayed on the *Saintpaulia* plants. The plants were placed in chambers where the light intensity and relative humidity were optimum for growth of the plant. The blossom-blight phase of the disease became evident 4 days or more after inoculation, depending upon the environmental factors.

The fungus was readily reisolated from lesions that resulted from inoculations; it was grown in culture and when the spores were used to inoculate healthy plants it again caused typical expression of the disease.

Isolates of *Botrytis* of the *cinerea* type from peony, tulip, and geranium also caused characteristic symptoms of the disease when inoculated into

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[8] Gloyer, W. O. Fungous diseases of the China aster. (Abstr.) Phytopath. **14**: 64. 1924.

[9] Baker, K. F. Observations on some Botrytis diseases in California. U. S. Dept. Agr., Plant Disease Reptr. 30: 145–155. 1946.

[10] The authors are indebted to Dr. C. J. Alexopoulos, Mycologist, Department of Botany and Plant Pathology, for the identification of the fungus.

Saintpaulia. Symptoms were indistinguishable from those caused by the isolate of *Botrytis cinerea* originally obtained from African violet tissue.

Undoubtedly one of the most efficient means of control for this disease is sanitation and adequate spacing of the *Saintpaulia* plants. Spacing allows ample air circulation and prevents the plants from touching each other. Good air circulation is very important, especially since authorities recommend low light intensity and a high relative humidity for the growth of the African violet. However, too high humidity and too low a light intensity, below approximately 450 foot-candles, should be avoided when practicable because these conditions favor the rapid spread of the fungus.

All diseased and dead tissue should be removed from the plants to prevent an accumulation of inoculum. The use of a sterile rooting medium for propagation and the use of "sterilized" potting soil is recommended to reduce the possibility of introducing inoculum from these sources. Care should be taken in handling the plants so as to avoid mechanical injury, thus limiting the number of avenues of entrance for the pathogen. The use of subirrigation might also aid in the control of the disease. Healthy, strongly growing plants are more resistant to *Botrytis* under many conditions than are weak, poorly growing plants.

Although no extensive search has been undertaken by the authors to ascertain the distribution of the disease, it can be assumed from conversation with scientists and growers that the disease may be widespread, and that it may appear wherever conditions are favorable. According to Dr. C. J. Alexopoulos, more than 20 years ago he noticed *Saintpaulia* plants in Illinois that had disease symptoms similar to those of plants which were inoculated with *Botrytis cinerea* by the authors. Professor R. A. Bowden, Department of Horticulture, stated that a disease of *Saintpaulia* not unlike the one described here is common in Georgia and that the use of glazed pots for growing the plants has reduced the occurrence of the disease there.

Because of the wide host range and prevalence of *Botrytis cinerea* it would be expected that the blossom and leaf blight of *Saintpaulia* may occur where conditions are favorable.—G. E. BECK, Department of Horticulture, and J. R. VAUGHN, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

Breaking of Cattleya Orchid Flowers by Orchid Mosaic Virus and its Transmission by Aphids.—A disease of Cattleya orchids which causes breaking in the color of the flowers (Fig. 1), distortion of the flowers, and mottling and malformation of the leaves has been noted by orchid growers in California for many years. Diseased plants typically become weak and stunted and produce progressively fewer flowers than normal.

The observations of some commercial growers in the San Francisco Bay region indicate that mosaic is present in some of the Cattleya plants imported from tropical America upon their arrival in California.

The writer has found no reference in the literature to a virus disease

of Cattleya orchids. There are, however, a few reports of diseases causing leaf mosaics in *Cymbidium*[1, 2, 3] and *Dendrobium*.[1, 4] Transmission has been reported only for Dendrobium mosaic and in this instance the disease was considered to be due to cucumber mosaic virus.[4] No statement has been published regarding the effects of mosaic on the flowers of *Cymbidium* and *Dendrobium*.

FIG. 1. *Cattleya mossiae* flower showing breaking in the color caused by orchid mosaic virus.

The present paper reports some of the results of experiments conducted during 1948 with a previously undescribed virus disease which causes a leaf mosaic and breaking in the flowers of *Cattleya* in California. The orchid plants used in these tests were *Cattleya mossiae* and *C. trianae* which are the species most commonly found to be diseased.[5]

Attempts to transmit the flower-breaking virus from orchid to orchid by

[1] Magee, C. J. Orchid mosaic. Australian Orchid Review **8**: 51–52. 1943.

[2] Bissett, J. The black spot or mosaic in Cymbidiums. Australian Orchid Review **10**: 48. 1945.

[3] Moore, W. C. Report on fungus, bacterial, and other diseases of crops in England and Wales for the years 1943–1946. Min. Agric. and Fish Bul. 139. 1947.

[4] Nobrega, N. R. Uma doenca de virus em orquidea. O Biologico **13**: 62. 1947.

[5] The writer wishes to acknowledge his appreciation to N. W. Curson of Oakland, California, and to the E. W. McLellan Company of San Francisco, for donating the experimental orchid plants used in this study; and to Mr. Willis Sibray of the Alameda County Agricultural Commissioner's office, whose cooperation facilitated the investigation.

juice inoculation have been unsuccessful. However, the green-peach aphid, *Myzus persicae* (Sulzer), proved to be a vector of the disease. The aphids used in these tests were reared on sugar beets. They were first transferred to the unopened buds or open broken flowers of diseased orchids where they were allowed to feed for from 24 to 48 hr., and then transferred by brush to the test plants. In two cases the transfer was made by caging aphid-infested inoculum on the healthy test plants. The number of aphids per test varied from 100 to 300. Both winged and wingless forms were used.

In eight tests infective aphids were caged on healthy flower buds within a day after the buds emerged from the bud sheaths. Seven of these plants have developed mosaic symptoms. Five of the seven plants so infected developed some breaking in the flowers initially infected and also later developed leaf symptoms. In two cases the flowers fed upon developed no symptoms, but new leaves, produced on these plants since infection, have developed symptoms.

The time between infection of the buds and expression of breaking in the color of the flowers which developed from those buds was surprisingly short, ranging from 12 to 19 days. The amount of breaking was relatively small in most of these flowers, sometimes being confined to one or two sepals or petals. This would be expected because of the short time elapsing between infection and opening of the flowers.

The time required for leaf symptoms to appear in plants which had been infected through the flower buds varied from $2\frac{1}{2}$ to $4\frac{1}{2}$ months depending upon the growing condition of the plants. In each case the symptoms were delayed until a new shoot developed.

Infective aphids were caged on the young leaves of three orchid plants which carried no flower buds. Transmission was accomplished in two of these tests. The third plant has made little growth since inoculation and may yet develop symptoms when new growth is produced. The first plant infected through the leaf was *Cattleya trianae*. Symptoms first appeared on the inoculated leaf 32 days after infection. A new shoot arising from a different part of the plant began to show symptoms 40 days after the test was begun.

No transmission was obtained in three tests in which infective aphids were caged only on the flower bud sheath, nor in two tests in which the bud sheath and a maturing leaf were the only tissues available to the infective aphids.

The identity of the virus has not yet been established. However, the possibility of its being spotted wilt virus is precluded by the fact that it is transmitted by aphids and because mechanically inoculated juice from leaves and flowers of mosaic orchids failed to produce symptoms in garden nasturtium (*Tropaeolum majus*), which is highly susceptible to spotted wilt virus. Other host range experiments, including tests with plants known to be susceptible to cucumber mosaic viruses, have thus far failed to indicate virus hosts other than Cattleya.

Work is still in progress on the virus-vector relationships of the disease and on the search for additional hosts of the virus.—D. D. JENSEN, Division of Entomology and Parasitology, University of California, Berkeley, California.

A Qualitative Chemical Test for Some Stone Fruit Virus Diseases.—In the course of biochemical and physiological investigations of virus diseases, a chemical test was developed which has been helpful in the diagnosis and study of some of the stone fruit virus diseases.[1] During 1948 a simpler, qualitative test was devised which has proven to be a valuable diagnostic tool, both in the field and in the laboratory. Leaf tissue is generally used for testing, but when cherry fruit tissue is used, this test appears to be specific for the "little cherry" complex.

The chemical test appears to be a measure of the disease intensity; or, in other words, a measure of the intensity in reaction between a virus and the host. The chemical substance or substances that are responsible for the color reactions have not been identified but are presumed to be polyphenols.

A leaf sample, obtained with the use of a paper punch that cuts a disk of tissue $\frac{1}{4}$ in. in diameter, is taken from the middle portion of a leaf, either directly through the midrib or halfway between the midrib and the leaf margin. The midrib sample is the more sensitive. A single disk of leaf tissue is then placed in a small test tube with 1 ml. of 4 per cent sodium hydroxide. The tube is heated over a flame until the contents have boiled for about 15 seconds. The tube is allowed to cool for 30 sec., and is then shaken very vigorously in order to incorporate air with the solution. Oxygen from the air serves as an oxidizing agent to produce the colors. Pink or red indicates a positive test; green, a negative test.

With sweet cherries the milder virus diseases, such as the "ring-spot" or "latent" group, give varying shades of pink or light red. The stronger diseases, such as little cherry, rusty mottle, or twisted leaf, give a deep red color. With peach leaf tissue only the stronger diseases give a positive test. In Washington, Western X disease is the only peach virus disease that falls in this class.

Care should be taken in sampling to avoid leaves from branches that have been girdled. Reddened areas on peach leaves resulting from such disorders as nitrogen deficiency, arsenic injury, or Coryneum blight may also give misleading results. When the cherry leaf samples are taken, only spur leaves should be used.

Cherry fruit tissue may also be tested. The only cherry virus disease that has been found to give a positive fruit test is "little cherry"; thus it seems the procedure is specific for this disease. A cube of tissue about 4 mm. to a side is cut from the cheek of the fruit and treated like the leaf

[1] Lindner, R. C. A rapid chemical test for some plant virus diseases. Science 107: 17–19. 1948.

tissue. Any shade of pink or red is indicative of a positive test for "little cherry." Yellow or brown indicates a negative test. The test works well on the fruit of the major commercially grown varieties of sweet cherries in Washington. The fruit, however, should be matured to the point that it is beginning to color or soften. Hard green fruit may give a false test. Fruit heavily infested with aphis, as well as fruit on a branch or spur that is girdled or infested with scale, should be avoided for samples. Certain Mazzard seedlings that produce small, bitter fruit also give a false test.

Samples of sour cherry fruit with typical "little cherry" symptoms give a distinctive red color when tested by this procedure. Occasionally a light pink is obtained with fruit that does not seem to have the "little cherry" disease. Since the "little cherry" virus is usually not uniformly systemic, comparative tests of healthy and diseased fruit from the same sour cherry tree should be made. A distinctive color difference between the healthy and diseased fruit samples indicates "little cherry."—R. C. LINDNER, Division of Plant Pathology, The State College of Washington, Tree Fruit Experiment Station, Wenatchee, Wash.; T. E. WEEKS, Washington State Department of Agriculture; and H. C. KIRKPATRICK, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Pythium Black Rot of Cattleya.[1]—Black rot of orchids has been described recently by Rosetti[2] in Brazil where it damages species of *Laelia*. Inoculations of an unidentified phycomycetous fungus isolated from diseased specimens into wounded pseudobulbs produced the disease in *L. purpurata* Lindl. and Paxt. and *L. crispa* Reichb. f. Limber,[3] in 1946, in his report on the distribution of black rot of orchids, stated that what appeared to be black rot was found on Cattleyas from England, Colombia, and Venezuela.

The disease was found on seedlings as well as on mature, flower-bearing plants of *Cattleya* sp. in greenhouses in California. A survey showed the disease to be widely distributed and, in some instances, taking a rather heavy toll of *Cattleya Fabia* Hort., *C. Forbesii* Lindl., *C. Mossiae* Hook., and *C. Trianae* Linden and Reichb. f. The condition has been known to orchid growers in California for some time and has always been associated with excessive watering, lack of ventilation, and low temperature.

Diseased seedlings growing in community pots damp off. A black discoloration appears in the growing point and the seedlings soon dry up completely (Fig. 1). The course of the disease is rapid and losses may be con-

[1] The authors wish to express their gratitude to Mr. W. S. Sibray of the Alameda County Agricultural Commissioner's Office for assistance in the studies presented in this paper.

[2] Rosetti, Victoria. Podridão preta das Orquideas. O Biologico 9: 201–205. 1943.

[3] Limber, D. P. A note on the distribution of black rot of orchids. U. S. Dept. Agr., Plant Dis. Rptr. 30: 89. 1946.

siderable. On the leaves of small individual plants, black spots of various sizes (Fig. 2, A) may coalesce and form larger, black, dead areas. In plants 2 or 3 years of age the disease may start in the root and spread toward the pseudobulb. The attacked roots die, and the pseudobulbs shrivel and finally lose all their contents. The disease frequently occurs after the plants are moved from one pot to another or when too much water is used. It also may start at the leaf tip and spread rapidly downward when atmospheric humidity is high (Fig. 2, E). In large plants a black, shiny discoloration appears on the leaf blade. Sometimes the tissues become soft and flaccid and on slight pressure water exudes in abundance. At this stage the leaves are somewhat translucent. In more advanced stages the tissues collapse and the translucency disappears. The pseudobulb between the

FIG. 1. Damping-off of Cattleya seedlings caused by *Pythium ultimum*. Severe damage in pot on the left.

crown and the leaf blade may become infected through either of these routes. Light-yellow to light-brown discolorations, in the form of streaks which enlarge longitudinally as well as transversely, soon change the pseudobulb to a black mass. The pathogen can spread from one pseudobulb to another until the whole plant loses its leaves and becomes a mass of mummified pseudobulbs.

Cultures of the pathogen were secured from damped-off Cattleya seedlings. Plants with black discoloration in the crown were pulled and the dead roots were cut off. Samples were washed in 6 changes of sterile distilled water, then placed on sterile paper in sterile Petri dishes in order that excess moisture might be absorbed. Subsequently the samples were cut into smaller pieces and transferred to acidulated potato-dextrose agar in a Petri dish. As soon as clean hyphae were seen under a dissecting micro-

scope, a piece of mycelium was transferred to potato-dextrose agar. In culturing the fungus from the pseudobulbs, these were split along the long

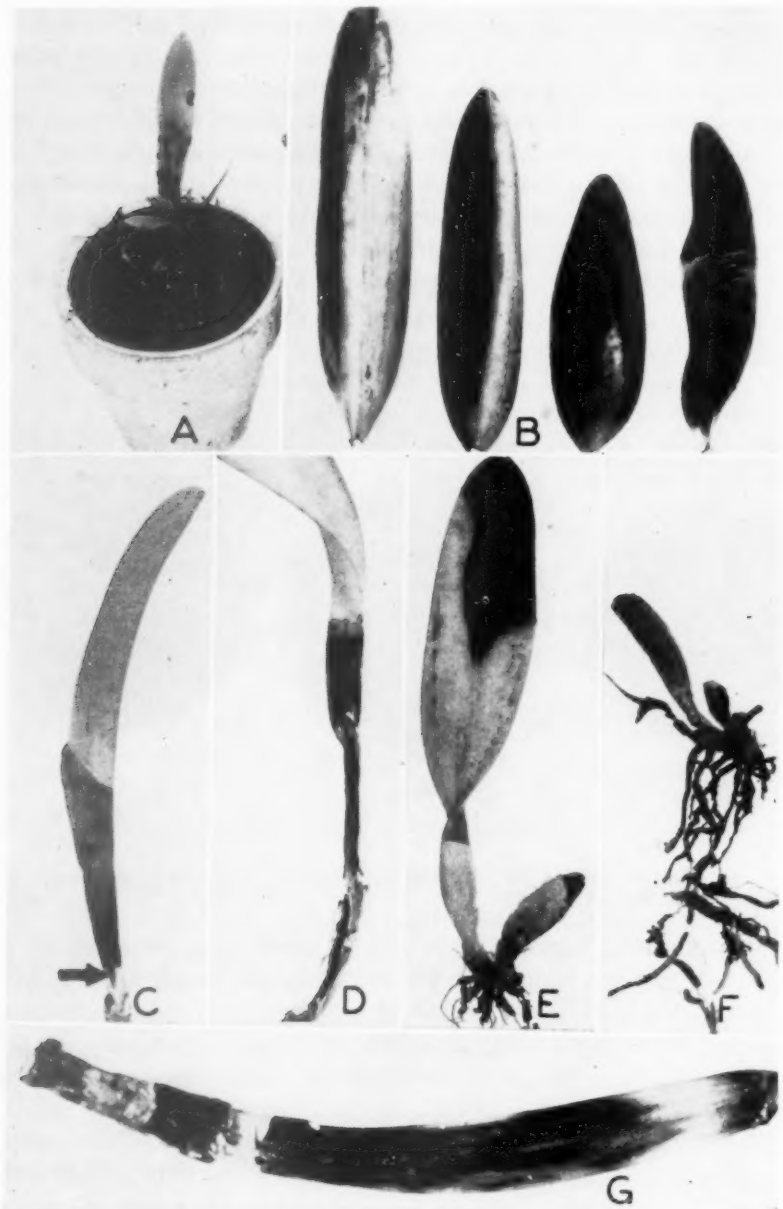


FIG. 2. Symptoms of Pythium black rot of orchid. A. Infections caused by zoospores of *Pythium ultimum* on leaf of *Cattleya Mossiae*. B. Infection on leaves of young Cattleya plants. The leaf on the right was soft and watery. C and D. Black rot produced by placing agar culture of the fungus on a pseudobulb. E. Infection of leaf tip and roots of *Cattleya* sp. from a commercial greenhouse. F. Severe black rot in a 5-year-old plant. G. Pseudobulb of *Cattleya Trianae* infected with *P. ultimum*.

axis and pieces of infected tissues were transferred, first to potato-dextrose agar acidified with lactic acid, and later to ordinary potato-dextrose agar. Leaf-blade tissues at the advancing margin of a lesion were used in the same way.

The fungus isolated conforms with *Pythium ultimum* Trow as defined by the junior author.[4]

Vegetative growth is abundant and arachnoid on media rich in carbohydrates. Fruiting structures are rarely produced on potato-dextrose agar. Reproductive bodies are produced in abundance on cornmeal agar and on plain water agar from potato-dextrose-agar blocks containing the fungus. Sporangia are mostly acrogenous on short, lateral branches, and measure 13.2 to 29.6 μ, average 21.9 μ, in diameter; intercalary sporangia 14.2 to 18.6 μ in width and 21.1 to 29.6μ in length occasionally occur.

The sporangia of *Pythium ultimum* commonly germinate by the production of germ tubes. Those of the orchid isolates were observed to germinate by the production of zoospores in a vesicle borne on a short emission tube. Although this manner of germination is rather unusual for sporangia in *P. ultimum*, it is not unique and cannot be considered a phenomenon alien to this species. Oogonia are similar to sporangia in disposition, shape, and size, and can only be distinguished from sporangia by the attendant antheridia and the oospores. Antheridia are monoclinous, and originate near the apex of the oogonial stalk; they are customarily sessile, short, inflated, and sharply upcurved, making apical contact with the basal portion of the oogonium. A fertilization tube is usually conspicuously present. The oospores are aplerotic, measure 19.4 to 23.9 μ, average 19.8 μ, in diameter, and are smooth and thick-walled, usually containing a single reserve globule and a subspherical refringent body.

Cardinal temperatures for vegetative growth of the orchid isolate are: minimum, 1° C.; optimum, 28° to 31° C.; maximum, 37° C. These values agree with those reported for *Pythium ultimum*.

To test the pathogenicity of the fungus, it was grown on potato-dextrose agar and small pieces were transferred to young pseudobulbs of *Cattleya Trianae*. These were kept at high humidity and a temperature of 65° to 75° F. Disease symptoms appeared after two days and the disease progressed rapidly (Fig. 2, C and D). *Pythium ultimum* was reisolated from infected plants. In some cases *P. ultimum* placed in a sterile watch glass with sterile water produced abundant zoospores; when these were sprayed on an unfolded leaf of a young plant, black spots developed on the leaf 5 to 8 days after spraying. This suggests the mode of spread of the fungus in orchid houses where overhead irrigation is practiced.

Inoculations of *Cattleya Fabia*, *C. Forbesii*, *C. Mossiae*, and *C. Trianae* resulted in typical symptoms. *Epidendrum O'Brienianum* Rolfe inocu-

[4] Middleton, John T. The taxonomy, host range, and geographic distribution of the genus Pythium. Mem. Torr. Bot. Club 20: 1–171. 1943.

lated with *Pythium ultimum* developed the disease. *Odontoglossum grande* Lindl. and *O. Schlieperianum* Reichb. f. failed to become diseased when inoculated with *P. ultimum*.

The seedling phase of the disease was controlled by watering the pots with a 1:2000 concentration of 8-hydroxyquinolin benzoate (Bioquin 700). The treatment can safely be repeated every week until no more disease appears. Watering the plants with a copper solution, 1:100,000 or 1:1,000,000, was also effective in stopping the disease. This treatment can be repeated if necessary but not often, because too much copper may accumulate in the Osmunda fiber. Control of the disease on large plants should be centered around reducing the humidity of the air, keeping the plants dry, and providing plenty of ventilation. Sometimes it may be necessary to expose diseased plants to the sun.—PETER A. ARK and JOHN T. MIDDLETON, Division of Plant Pathology, University of California, Berkeley and Riverside, California.

Resistance in Alfalfa to Yellow Leaf Blotch. Resistance in alfalfa to yellow leaf blotch caused by *Pseudopeziza jonesii* (Fckl.) Nannf. has been observed in the nursery in two groups of selected plants. The first noted were two clons among four derived from survivors selected in 1944 from a nursery row seeded with alfalfa, Conference Number A 31. These clons first attracted attention because of resistance to *Pseudopeziza medicaginis* (Lib.) Sacc. The second group of plants were among selections from a field of Kansas common alfalfa. This field, seeded in 1947, attracted attention in the autumn of that year because it furnished an unusual opportunity to select plants resistant to *Pseudopeziza medicaginis*. The growth of this ample but not overly dense stand had been restricted by summer drought, and the autumn growth bore common leaf spot in great abundance mixed with only a little of the leaf spot caused by *Pleospora herbarum* (Pers. ex Fr.) Rab. Twenty-one plants with clean foliage were obtained from this field. The frequency of such plants was estimated to be of the order of hundredths of one per cent.

Not all of these selections proved in greenhouse tests to be very highly resistant to leaf spot, and a few were so poor in type that they were of little interest. However, in the spring of 1949 fourteen of these plants were represented in the nursery by clons and by small populations obtained by selfing. Leaf spot did not develop in the first growth in this nursery until very late, and even then in very small amount; but yellow leaf blotch did develop early in moderate amount, apparently from a single short period of infection. In a few clons that appeared to be especially susceptible to injury from yellow leaf blotch the lower leaves had fallen by the middle of June. Among the fourteen clons and selfed progenies resistant to leaf spot, four retained lower leaves almost free from yellow leaf blotch, as late as June 23. On June 29 well-developed blotch lesions were found on lower leaves of two of the four clons. The nursery was cut shortly after this.

Thus it appears that in at least two of the Kansas selections there is a type of resistance to yellow leaf blotch similar to a type of resistance to leaf spot in which young leaves are not infected readily or abundantly, but in which the fungus may survive and produce at least a few lesions in old leaves if they are retained on the plant.

Unfortunately it does not appear possible to test this apparent resistance immediately by controlled artificial inoculations. Suitable fruiting cultures have not been produced, but the use of apothecia collected in the field remains to be explored. Even with these handicaps, it appears highly desirable to seek new selections whenever opportunity offers, especially in wilt-resistant stocks.—FRED REUEL JONES, U. S. Department of Agriculture, Madison, Wisconsin.

A Mass-Production Method for Studies of Barley Smuts in the Greenhouse.[1]—With a view to utilizing greenhouse space as efficiently as possible in studies of the barley smuts, the writer has developed methods whereby from 30,000 to over 60,000 barley plants can be grown each year during the winter months in a greenhouse bench area of 420 sq. ft. About 8,000 to 10,000 plants would be grown by former methods.

Seeding directly in greenhouse benches often results in thin irregular stands, sometimes as low as 50 to 75 per cent, due to poor seed germination or to smut infection of emerging seedlings. To overcome this, H. A. Rodenhiser, one of the writer's associates, devised the method of seeding in soil in 4 × 8-in. tin pans, 2 in. deep. The pans are placed in covered tin boxes to check evaporation during seed germination. Moistened blotters usually are put in the tin boxes to maintain the humidity. The seedlings are transplanted to greenhouse benches when they are about 1 in. high. This method of germinating seed in chambers or rooms with controlled temperatures optimum for infection is now generally used by cereal pathologists at the Plant Industry Station, Beltsville, Maryland. The accurate control over temperature during the critical period of seed germination and the efficient use of greenhouse space resulting from full stands of plants fully justify the additional work involved.

Another step toward economy resulted from the discovery that under usual greenhouse conditions, small plants with a single culm 12 to 18 in. high served as well as much taller plants with numerous culms for indicating smut resistance or susceptibility.[2, 3] Barley plants of this size can be grown from spacing as close as 1 in. in rows only 4 in. apart. One hundred per cent smutted heads frequently has been obtained in the 4-in. plantings.

[1] The barley smuts involved were the covered smut (*Ustilago hordei* (Pers.) Lagerh.), the nuda loose smut (*U. nuda* (Jens.) Rostr.), and the nigra loose smut (*U. nigra* Tapke).

[2] Reed, G. M. Influence of growth of the host on smut development. Proc. Amer. Phil. Soc. 79: 303–326. 1938.

[3] Tapke, V. F. Influence of varietal resistance, sap acidity, and certain environmental factors on the occurrence of loose smut in wheat. Jour. Agr. Res. [U.S.] 39(5): 313–339. 1929.

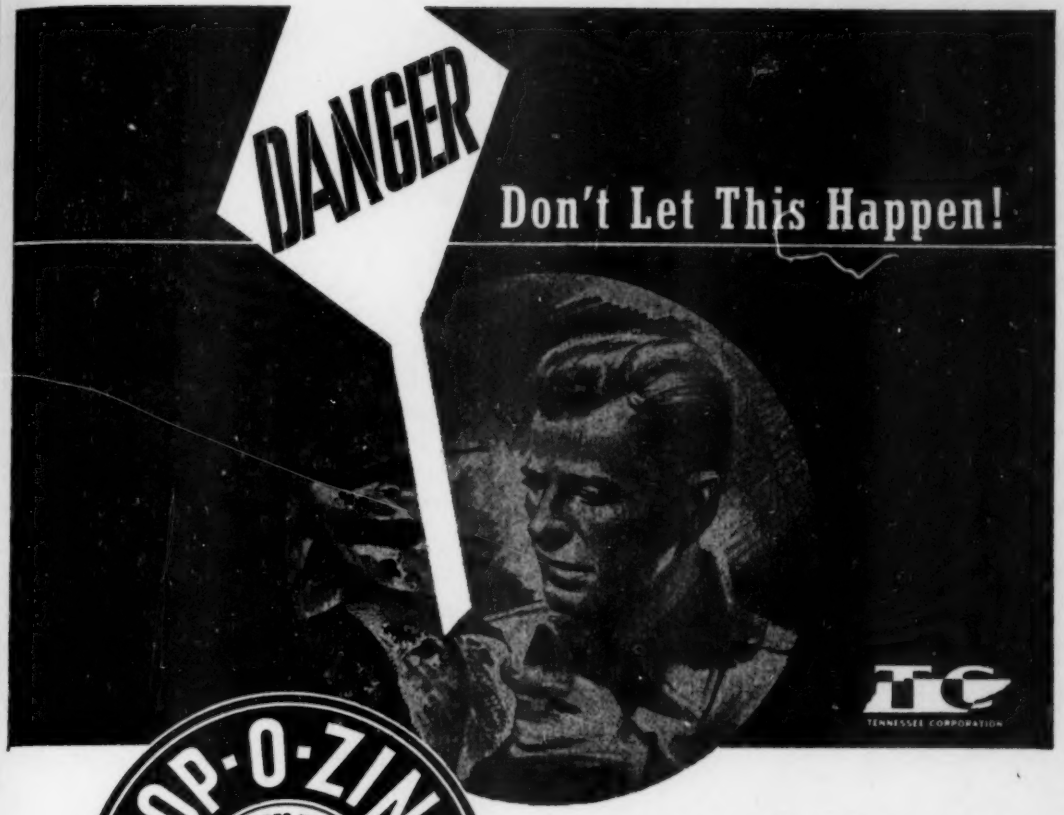
Spacings 6 to 8 in. between rows formerly were used. Plant growth is restricted also by light watering two or three times a week instead of daily. In one experiment barley plants were grown 1 in. apart in rows only 2 in. apart. Up to 83 per cent smutted heads were obtained on susceptible varieties. The writer would hesitate to recommend this spacing without qualification but the experiment indicates the possibility of very thick planting.

Economy also resulted from the practice used by J. Allen Clark of growing two crops of wheat per year instead of the usual one crop in the greenhouse at Beltsville, Maryland. The first crop was sown September 10. From mid-October until maturity in November or December the plants received artificial light from 5 P. M. to 11 P. M. daily. Four 100-watt mazda bulbs under reflectors were used for a bench $17\frac{3}{4}$ by $4\frac{3}{4}$ feet. The bulbs were spaced equally over the long axis of the bench and hung approximately 1 ft. above the top of the plants. A second crop of wheat sown in January matured in March or April without supplemental artificial light. The writer has used this two-crop system during the past 3 years. It works very well with spring barleys grown from smutted seed and it doubles the capacity of the greenhouse space.

On the basis of their studies on photoperiodicity, Borthwick *et al.*[4] suggested to the writer that he might effect economy without loss of efficiency by using daily supplemental lighting from 12 midnight to 2 A. M. instead of from 5 P. M. to 11 P. M. During the 2 years that the writer has tried this the plant growth and the incidence of smut have been satisfactory.

Some studies of small-grain smuts require a great amount of tedious work in processing the seed. When the test is conducted in the field, the hazard of conditions unfavorable to infection is a constant menace and it may be necessary to repeat the processing several times or more before conditions satisfactory for good infection are obtained. When the seed is sown under the controlled conditions of a greenhouse, however, the success of the test is practically certain. This seems to add incalculably to the value of a mass-production method of greenhouse testing.—V. F. TAPKE, Senior Pathologist, Division of Cereal Crops and Diseases, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

[4] Borthwick, H. A., S. B. Hendricks, and M. W. Parker. Action spectrum for photoperiodic control of floral initiation of a long-day plant, Wintex barley (*Hordeum vulgare*). Bot. Gaz. 110: 103–118. 1948.



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SEED Treating NEWS FROM DU PONT

"ARASAN" FOR GLADIOLUS CORM ROT

"Tests of nine chemical treatments to control gladiolus corm rot at Colorado A & M College Experiment Station have shown "Arasan" applied full strength as a dust to be the best," says the monthly *Colorado A & M News*.

The Colorado test was one of eight conducted simultaneously in different states, to find the best control of Fusarium rot. The Picardy variety was used. Results were judged on the basis of number of plants which emerged; total plants flowering; total corms harvested; number of rot-free corms harvested; weight of rot-free corms harvested; number of corms having Fusarium rot; and number of corms showing evident bacterial scab.

COTTON YIELDS INCREASED IN TEXAS TESTS

In a four-year test by the Texas Agricultural Experiment Station with chemical seed treatment for cotton, results showed good increases. There was a 25 per cent increase at College Station, a 15 per cent increase at Temple, a 10 per cent increase in the Brazos bottoms near College Station, and a 4 per cent increase in the average yield at Lubbock.

In another test at the Temple Sub-station, treated seed gave 25 to 30 per cent better stands than untreated seed. At the same time, the plants made greater yields per acre, and had less angular leaf, spot. The difference in yields between treated and untreated seed varied from year to year, depending on the amount of disease germs on the seed and the condition of the soil at planting time, according to E. A. Miller, Extension Agronomist at Texas A & M College.

SAFFLOWER SEED SHOULD BE TREATED

Safflower, the new oil-seed crop that is taking hold in the dry areas of Nebraska and Colorado, should be treated with seed disinfectant before planting. "Ceresan" seed disinfectant is recommended by Dr. Carl E. Claassen, of the University of Nebraska, for use at the rate of one to two ounces per bushel. This treatment helps to insure uniform stands, and to reduce the number of primary sources of leaf rust infection. Unlike cereal rusts, safflower leaf rust is also transmitted on the seed.

Safflower is grown primarily for the oil that is extracted from the seed, and is especially adapted to areas of low humidity. The by-product is a high-protein feed that compares with other oil-seed meals. Safflower oil is considered especially suitable for high-quality paints.

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